

GROWTH AND DEVELOPMENT AT THE SHOOT APEX  
OF IMPATIENS BALSAMINA L. DURING FLOWERING  
AND REVERSION

BY

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Abbreviations

ABA	abscisic acid
AMPS	10% (w/v) ammonium persulphate
aq.	aqueous
$^{\circ}\text{C}$	degrees Celsius
cm	centimetre
d	day
dm	decimetre
DNP	day neutral plant
DOC	0.15% (w/v) sodium deoxycholate
EB	extraction buffer
EDTA	ethylenediaminetetracetic acid
g	gram
$\underline{g}$	acceleration due to gravity (relative centrifugal force)
G.C.R.I.	Glasshouse Crops Research Institute
h	hour
kg	kilogram
kV	kilovolts
LD	long day (see p. 17 )
LDP	long day plant
LM	light microscopy
$\log_{10}$	logarithm to the base 10
$\log_e$	logarithm to the base e
mg	milligram
$\mu\text{g}$	microgram
M	molar
$\mu\text{mol}$	micromole
ml	millilitre



$\mu$ l	microlitre
mm	millimetre
$\mu$ m	micrometre
min	minute
n	number of replicates
nm	nanometre
n.s.	not significant
%	per cent
P	probability
r	the correlation coefficient
$\bar{r}$	the plastochron ratio
RNA	ribonucleic acid
R0-R7	reversion types (see Table 6, p. 60 )
SB	sample buffer
SD	short day (see p. 17 )
SDP	short day plant
SLDP	short long day plant
s.e.	standard error of the mean
sec	second
SEM	scanning electron microscope
SLS	sodium lauryl sulphate (= sodium dodecyl sulphate)
Stereological symbols	see pages 40-43
TCA	72% (w/v) trichloroacetic acid
TEM	transmission electron microscopy
TEMED	N,N,N',N'-tetramethylethylenediamine
V	volt



<u>v</u>	the relative distance of any two successive primordia from the apical surface
V/V	volume per unit volume
vol.	volume
W/V	weight per unit volume
W	watt
≥	greater than or equal to
>	greater than
≤	less than or equal to
<	less than
2:3, 3:5, 5:8 etc	parastichy numbers; symbols are those used by Richards (1951, p.517)

#### Definitions

Divergence angle	The angle at the centre of the apex between two successively initiated primordia
Frustum	The stem tissue adjacent to the primordium, and the stem tissue between the abaxial insertion point of the primordium and the adaxial insertion point of the next oldest primordium
Genetic spiral (=generative spiral)	The spiral that can be traced between successively initiated primordia
Plastochron	The interval of time between the initiation of successive primordia by the apical meristem



Plastochron ratio	The ratio between the transverse distance of the primordium from the apical centre relative to that of the next primordium to be produced
Primordium	The part of the shoot apical meristem that develops into a leaf, flower part, or other lateral appendage. A primordium was considered to be present when a distinct bump was visible on the apical surface, in photographs or in dissections of the shoot apex; it was recognised in transverse sections as a more deeply-staining area of cells on the apical surface



CONTENTS

	<u>PAGE</u>
Title page .. .. .	i
Declaration .. .. .	ii
Acknowledgements .. .. .	iii
Abbreviations .. .. .	iv
Definitions .. .. .	vi
 Contents	 viii
Abstract	xiv
Chapter 1: Introduction .. .. .	1
Chapter 2: Materials and Methods .. .. .	16
2.1 Plant material .. .. .	17
2.2 Growth conditions .. .. .	17
2.3 Seed germination, growth of plants and selection for developmental uniformity ..	17
2.4 Treatments causing flowering and reversion .. .. .	18
2.5 Selection for uniformity or reversion response .. .. .	18
2.6 Growth of the apex and primordia .. .. .	19
2.6.1 Number of leaves plus primordia	19
2.6.2 Developmental stages .. .. .	20
2.6.3 Distances of primordia below the apical surface .. .. .	20
2.6.4 The plastochron ratio .. .. .	24
2.6.5 Area and vertical relative growth rates per day of the apex .. .. .	27
2.6.6 Apical shape .. .. .	27



	<u>PAGE</u>
2.6.7 Lengths and widths of primordia ..	27
2.6.8 Areas of mature parts occupied by petal pigment .. .. .	28
2.7 Stereological analysis of developing primordia .. .. .	29
2.7.1 Tissue preparation .. .. .	29
2.7.2 Sectioning and staining of primordia .. .. .	30
2.7.3 Photography of the sectioned primordia; location of areas for stereological analysis .. ..	33
A. Light microscope photographs	33
B. Transmission electron micro- scope photographs .. .. .	34
2.7.4 Analysis of the photographs ..	36
Level 1 .. .. .	36
Level 2 .. .. .	39
2.7.5 Analysis of the data .. .. .	39
Level 1 .. .. .	39
Level 2 .. .. .	41
2.8 Gel electrophoresis .. .. .	43
2.8.1 Extraction and precipitation of protein .. .. .	43
A. Mature leaves and petals ..	44
B. Leaf, petal and reverted part primordia .. .. .	47
2.8.2 Preparation, running and staining of gels .. .. .	50



	<u>PAGE</u>
Chapters 3-6: Results .. .. .	53
3 Development of the experimental system ..	54
3.1 Obtaining developmental uniformity ..	54
3.2 The response to SD and LD .. .. .	56
3.3 Improving the uniformity of the reversion response .. .. .	61
3.3.1 The effect of altering the growth conditions .. .. .	61
3.3.2 The relationship between flower colour and reversion response; its use in obtaining uniform reversions	65
4 Flowering in SD and reversion in 5SD+LD ..	69
4.1 Morphological characteristics of flowering and formation of the R4 reversion type .. .. .	69
4.1.1 The terminal meristem .. .. .	69
4.1.2 The axillary meristems .. .. .	73
4.2 Changes in apical growth and phyllotaxis	74
4.2.1 Rates of primordium initiation ..	74
4.2.2 Divergence angles .. .. .	80
4.2.3 Area of the apical surface and areas of primordia at initiation	86
A. Early flower development and reversion (days 0-12) .. .. .	86
B. Later development of the flowering and reverted apices (days 11-21)	88
C. The mature reverted (5SD+LD) and vegetative (LD) apices (days 70-74) .. .. .	94



	<u>PAGE</u>
4.2.4 Lengths of stem frusta on	
initiation .. .. .	94
A. Early flower development and	
reversion (days 0-12) .. .	94
B. Later development of the flower-	
ing and reverted apices (days	
11-21) .. .	102
C. The mature reverted (5SD+LD)	
and vegetative (LD) apices	
(days 70-74) .. .	109
4.2.5 Area and vertical relative growth	
rates per day of the apex .. .	112
A. Early flower development and	
reversion (days 0-12) .. .	112
B. Later development of the flower-	
ing and reverted apices (days	
11-21) .. .	112
C. The mature reverted (5SD+LD)	
and vegetative (LD) apices	
(days 70-74) .. .	117
5 Flowering of the reverted meristem on return	
to SD .. .. .	119
5.1 Morphological changes on flowering of	
the reverted meristem .. .	125
5.2 Changes in apical growth and phyllotaxis	137
5.2.1 Rates of primordium initiation .. .	137
5.2.2 Divergence angles .. .	140
5.2.3 Area of the apical surface and	
areas of primordia at initiation	143



	<u>PAGE</u>
5.2.4 Lengths of stem frusta at initiation .. .. .	149
5.2.5 Area and vertical relative growth rates per day of the apex .. ..	160
6 Development of the primordia under different photoperiods .. .. .	165
6.1 The origin of intermediate parts: is their form determined at initiation, or does it result from a switch from petal to leaf development after primordium initiation? .. .. .	166
6.1.1 Comparative analysis of the changes in shape that occur during the growth of leaf, petal, intermediate part, bract, and reverting bract primordia .. .. .	166
6.1.2 Gel electrophoretic study of proteins in leaves, petals and intermediate parts .. .. .	190
6.2 The time of commitment of primordia (to leaf development) on the reverted (5SD+LD) apex. Stereological analysis of cellular changes occurring at the time of commitment .. .. .	194
Chapter 7: Discussion .. .. .	227
7.1 Changes in apical growth and phyllotaxis on flowering, reversion and re-flowering	228
7.1.1 Are evocational changes in apical growth and phyllotaxis essential for flower part initiation? .. ..	228



7.1.2	If the reverted apex is partially evoked, are the leaves of the reverted plant also partially induced? .. .. .	232
7.1.3	How are the changes in phyllotaxis and apical growth controlled? ..	235
A.	The change from spiral to whorled or pseudo-whorled phyllotaxis: a decrease in divergence angle?	235
B.	Mechanisms involved in the transitions to and from whorled or pseudo-whorled phyllotaxis on flowering and reversion .. ..	244
	<u>The role of the number of primordia on the apical surface and stem frustum length at initiation .. .. .</u>	244
	<u>The importance of changes in the ratio of primordium to frustum size at initiation in determining changes in the number of primordia on the apical surface ..</u>	247
	<u>The sequence of changes on flowering and reversion; a possible role for internode growth in controlling phyllotaxis ..</u>	253
C.	Summary of possible mechanisms involved in the changes in phyllotaxis on flowering and reversion; unanswered questions .. ..	260



	<u>PAGE</u>
7.1.4 The control of final internode length .. .. .	262
7.2 Development of the primordia on flowering, reverting and re-flowering apices .. ..	264
7.2.1 The relationship between primord- ium development and apical devel- opment .. .. .	264
7.2.2 Primordium commitment and its control .. .. .	266
A. Commitment of primordia in <u>Impatiens</u> is unusual because it does not occur at the level of the whole organ, and is very late .. .. .	266
B. What does commitment in <u>Impatiens</u> primordia involve? .. .. .	270
7.2.3 Commitment of the primordia and commitment of the apex .. ..	276
A. Two types of commitment reveal- ed by <u>Impatiens</u> .. .. .	276
B. What is the nature of commitment of the apex to flower? .. ..	278
7.3 Critical areas for future work on flower- ing; the value of <u>Impatiens</u> in this work	282
7.3.1 The component processes of induction and evocation .. ..	282
7.3.2 Commitment - a key event? .. ..	285
References .. .. .	287



Appendix 1:	Plants showing inflorescence	
	reversion .. .. .	308
Appendix 2:	A) Composition of Edinburgh form-	
	ulation of G.C.R.I. potting	
	compost number 1 .. .. .	316
	B) Composition of Hoagland's	
	solution as used for liquid	
	feed .. .. .	316
Appendix 3:	Published paper .. .. .	318



# ABSTRACT

When plants of Impatiens balsamina L. were subjected to 5 short days and then replaced in long days, they began to form a terminal flower and then reverted to vegetative growth at this terminal shoot apex. The onset of flowering was accompanied by an increase in the rate of initiation of primordia, an increase in the growth rate of the apex, a change in primordium arrangement from spiral to whorled or pseudo-whorled, a lack of internodes, and a reduction in the size at initiation of the primordia and also of the stem frusta which give rise to nodal and internodal tissues. On reversion, parts intermediate between petals and leaves were formed, followed by leaves, although in reverted apices the size at initiation and the arrangement of primordia remained the same as in the flowering apex for at least 20 days after transfer to long days. The apical growth rate and the rate of primordium initiation decreased on reversion to leaf formation, but remained higher than in the original vegetative apex for about 15-20 days after transfer to long days. Eventually the growth pattern and phyllotaxis of the reverted apex returned to that of the original vegetative apex. Since the changes in apical growth and phyllotaxis which occur on the transition to flowering are not reversed at the same time as reversion to leaf formation, the development of organs as leaves or petals is not directly related to the growth rate of the apex, or the arrangement, rate of initiation or size at initiation of primordia.



In further experiments plants were given 5 short days, then 9 long days, and then short days again. On re-transfer to short days petal initiation began at once and small primordia already present at that time developed as petals, instead of as leaves. However, the primordia became progressively committed to leaf development as their length increased from 0 to 750  $\mu\text{m}$ . Commitment paralleled cell vacuolation, but whereas commitment occurred earlier in the tip than in the base of the primordia, vacuolation did not. The rates of primordium initiation and apical growth, the primordium arrangement, and the sizes at initiation of the primordia and the stem frusta, all remained the same, on re-transfer to short days, as in plants left in long days. This absence of change and the increased reactivity of reverted plants to short days (compared with vegetative plants) suggests that in reverted plants flowering remains partially evoked, and that the changes in apical growth and phyllotaxis needed for flowering have already been achieved.

It is suggested that the observed changes in phyllotaxis may be governed by the sizes of the primordia and frusta at initiation, and the rates at which these sizes change relative to one another.



CHAPTER 1  
INTRODUCTION



## CHAPTER 1: INTRODUCTION

Early research into the control of flowering showed that many species are induced to flower by specific photoperiodic conditions (Garner and Allard, 1920; see Vince-Prue, 1975 for review). As a result of this and more recent work, much is now known about the characteristics of the phytochrome-mediated<sup>d</sup> flowering response in photoperiodically sensitive plants (see Hillman, 1967; Evans, 1971; Vince-Prue, 1975, 1983). However, it is still unclear how the perception of inductive photoperiods by the leaves results in the formation of the flower at the shoot apex (Bernier, Kinet and Sachs, 1981a and b). Promotive effects in inductive conditions (Zeevaart, 1984) and inhibitory effects in non-inductive conditions (Schwabe, 1984a) are both thought to be involved, but the nature of the floral stimulus remains elusive (see Lang, 1965; Evans, 1969, Zeevaart, 1976).

The intransigence of the problem of the nature of the floral stimulus has led to a shift in the emphasis of some of the work on flowering physiology, to consideration of the events occurring in the shoot apex on arrival of the floral stimulus. These events constitute the process of floral evocation, and lead to flowering (Evans, 1969). Much detailed information has now been gathered on the cellular and morphological events that characterize evocation in several species (Bernier et al, 1981b). Changes at the cellular level include an increase in the rate of RNA synthesis in Lolium temulentum (Rijven and



Evans, 1967; Knox and Evans, 1968; Evans, Knox and Rijven, 1970), Sinapis alba (Bronchart, Bernier, Kinet and Havelange, 1970; Pryke and Bernier, 1978), and Pharbitis nil (Gressel, Zilberstein and Arzee, 1970; Arzee, Zilberstein and Gressel, 1975; Gressel, Zilberstein, Strausbauch and Arzee, 1978), and in the RNA content of the cells of the apical meristem of Sinapis (Jacqmard, Miksche and Bernier, 1972), Chenopodium rubrum (Seidlova, 1974), and Silene coeli-rosa (Miller and Lyndon, 1977), or the cells of the potential spikelet primordia in Lolium (Knox and Evans, 1966). The increase in concentration of ribosomes seen in electron micrographs (Nougarède, 1967) reflects the increase in RNA and protein content that occurs during evocation. An increase in the mitotic index has been detected during evocation of the apical meristem of Xanthium strumarium, Chenopodium album, Pharbitis, Sinapis (see Bernier, 1971) and Silene (Francis and Lyndon, 1979). In Sinapis, Xanthium and Silene this is associated with an increase in the degree of synchrony in cell division (Bernier *et al.*, 1981b; Jacqmard, Raju, Kinet and Bernier, 1976; Francis and Lyndon, 1979; Grose and Lyndon, 1984). An increase in the relative growth rate of the apical meristem is also found in most species just before or at flower initiation (Sunderland, 1961; Corson, 1969; Saint-Côme, 1971; Bodson, 1975; Miller and Lyndon, 1975, 1976; see Lyndon and Francis, 1984, for review).

At the morphological level, the apex enlarges on flowering in most, though not all, species which have been



investigated (Table 1). A change in phyllotaxis, consistent with a decrease in the size of the primordia at initiation (relative to the size of the apical dome) has been reported in Chrysanthemum morifolium (Evington, 1955; Schwabe, 1959), Xanthium (Erickson and Meicenheimer, 1977), Silene (Lyndon, 1978), Ranunculus repens (Meicenheimer, 1979), and Epilobium hirsutum (Meicenheimer, 1982). The rate of primordium initiation typically increases at the transition to flowering (Table 2). It is worth noting, however, that in Bellis perennis and Chenopodium amaranticolor there may be a pronounced delay in primordium initiation during the earliest stages of the floral transition, before any marked increase in initiation rate associated with flowering itself (Philipson, 1946; Thomas, 1961a).

To understand how flowering is controlled, it is necessary to know how the events of evocation are linked to the subsequent initiation of flower parts. Even where an evocational event is considered essential to flowering, as for instance cell synchronisation is in Sinapis (Bernier, Kinet, Bodson, Rouma and Jacqmard, 1974), the mechanism by which it causes flowering is unknown. This is because evocation is sometimes defined as the events in the apex that lead to commitment to flowering (Bernier, 1979), and the nature of the commitment step is unknown. The time of commitment to flowering has been established in some plants, such as Sinapis in which a 'point of no return' can be demonstrated about 44 h after the start of the inductive long day (Kinet, Bodson,



TABLE 1. Changes in the size of the shoot apex on flowering

Apex	Species	Reference
Increases in size	<u>Bellis perennis</u>	Philipson, 1946
	<u>Chrysanthemum morifolium</u>	Evington, 1955; Schwabe, 1959; Horridge and Cockshull, 1979
	<u>Aster sinensis</u>	Lance, 1957
	<u>Lolium temulentum</u>	Evans, 1960
	<u>Chenopodium amaranticolor</u>	Thomas, 1961a
	<u>Perilla frutescens</u> (green- leaved)	Jacobs and Raghavan, 1962
	<u>Sinapis alba</u>	Bernier, Kinet and Bronchart, 1967
	<u>Triticum aestivum</u>	Kirby, 1974
	<u>Silene coeli-rosa</u>	Lyndon, 1977
	<u>Xanthium pennsylvanicum</u> (=X. strumarium)	Erickson and Meicenheimer, 1977
	<u>Ranunculus repens</u>	Meicenheimer, 1979
	<u>Epilobium hirsutum</u>	Meicenheimer, 1982
	<u>Stylosanthes guianensis</u>	Ison, 1984
	<u>Cannabis sativa</u>	Heslop-Harrison and Heslop-Harrison, 1958
	<u>Perilla nankinensis</u> (red-leaved)	Nougarède, Bronchart, Bernier and Rondet, 1964
Decreases in size	<u>Humulus lupulus</u>	Thomas and Schwabe, 1970



TABLE 2. Species in which the rate of primordium initiation increases on flowering

Species	Reference
<u>Chrysanthemum morifolium</u>	Schwabe, 1959; Horridge and Cockshull, 1979
<u>Chenopodium amaranticolor</u>	Thomas, 1961a
<u>Secale cereale</u> )	Sunderland, 1961
<u>Lupinus albus</u> )	Jacobs and Raghavan, 1962
<u>Perilla frutescens</u> (green-leaved)	
<u>Glycine max</u> )	
<u>Kalanchoe blossfeldiana</u> )	
<u>Perilla nankinensis</u> (red-leaved) )	
<u>Xanthium pennsylvanicum</u> (=X. strumarium) )	
<u>Antirrhinum majus</u> )	Langer and Bussell, 1964
<u>Arabidopsis thaliana</u> )	
<u>Plantago lanceolata</u> )	
<u>Rudbeckia hirta</u> )	
<u>Vicia faba</u> )	
<u>Fagopyron tartaricum</u> )	
<u>Malus domestica</u>	Fulford, 1965
<u>Pharbitis nil</u>	Wada, 1967
<u>Nicotiana tabacum</u>	Hopkinson and Hannam, 1969
<u>Triticum aestivum</u>	Kirby, 1974
<u>Silene coeli-rosa</u>	Miller and Lyndon, 1976
<u>Hordeum vulgare</u>	Dale and Wilson, 1979
<u>Ranunculus repens</u>	Meicenheimer, 1979
<u>Epilobium hirsutum</u>	Meicenheimer, 1982



Alvinia and Bernier, 1971). However, the links between cell synchronisation, the events at the 'point of no return' and the events of flower bud initiation, remain completely unknown.

In plants in which reversion of the flower meristem to vegetative growth can occur after flower formation has started, it is obvious that there is no 'point of no return' before flower development begins. This suggests that in such plants there is no commitment to flower. In these cases there is therefore no reason to suppose that events occurring in the apex before flowering (but after transfer to inductive conditions) are of any more (or less) significance than those occurring during flowering, since reversion to vegetative growth could presumably involve changes in either or both types of events. Throughout this thesis I shall therefore use evocation to mean: the response of the shoot apex to inductive signals. This very neutral definition includes any events at the apex which are associated, not only with the initial transition to flowering, but also with the maintenance of the flowering state. Hence, it allows for the possibility that, in plants that can revert, evocation and flower formation may proceed simultaneously; we can then ask whether there is a direct relationship between the events of evocation and the events of flower formation in these cases. Furthermore, it should be possible to see whether reversion to vegetative growth involves reversal of all preceding evocational changes, or whether the apex that results is partially evoked. The



response of a partially evoked apex to further induction should allow us to pinpoint which subsequent evocational changes correspond to particular events of flower formation.

From these considerations it will be clear that reversion, reflecting as it does an absence of commitment to flower, offers the opportunity to study the direct effects of evocation on the process of flowering itself. The aim of the work reported in this thesis was therefore to carry out an experimental study of flowering and reversion of the shoot apex. The first problem was to select the plant material most suitable for this work.

In its broadest sense the term reversion can be used to include a wide variety of abnormal types of inflorescence and flower development. In many cases the apical meristem itself does not revert to vegetative growth, but the flowers initiated either fail to develop or else take on vegetative characteristics. Examples of this occur in plants requiring a particular daylength regime for flower development. In Chrysanthemum morifolium, plants grown in long days initiate an inflorescence which fails to develop. If the inflorescence is forced to develop, by removing either the lateral branches which grow out below it or the inflorescence itself and rooting it separately, bracts are formed on the capitulum. Shoots also frequently grow out from the florets to give secondary inflorescences (Schwabe, 1951). In Cosmos sulphureus (Biddulph, 1936) and Rudbeckia bicolor (Murneek, 1940), transfer to non-inductive conditions during inflorescence development causes bracts to become foliaceous, and leafy



shoots to grow out below the inflorescence. Similarly, after a minimal inductive treatment Kalanchoë blossfeldiana may form a vegetative inflorescence (Harder, 1948). Transfer of the LDP Triticum aestivum to short days during inflorescence development causes the lemmas and glumes to become leafy; a secondary inflorescence occasionally grows out of the ovary if transfer to short days occurs after stamen initiation (Fischer, 1972). Various flower abnormalities, and secondary inflorescence formation, in Dianthus caryophyllus may be due to changes in daylength (Blake, 1962). Infection by viruses (Kunkel, 1953), or mycoplasmas (Daniels, 1979) can cause teratological structures (leafy petals, petaloid stamens etc.) within the flower that resemble those found in flower reversions (see p.274). In other cases flower abnormalities apparently arise spontaneously (see Meyer, 1966, for review). However, in all these cases there is little evidence that the apex itself resumes vegetative growth after having begun to flower. A useful discussion of the implications of teratological phenomena can be found in Heslop-Harrison (1952).

Another type of reversion is found in those grasses which show vegetative proliferation of the inflorescence. This may occur in response to changes in photoperiod, as in Cynosurus cristatus (Wycherly, 1952, 1954; Evans, 1960), Lolium perenne (S23) (Evans, 1960), and Phleum pratense (S48) (Langer and Ryle, 1958), or it may be genetically determined, as in Festuca vivipara, Poa alpina var. vivipara and others (Langer and Ryle, 1958). However, it results



from the development of a vegetative shoot at the side of the normal reproductive structure, rather than from reversion of the reproductive apex to vegetative growth (Langer and Ryle, 1958; Evans, 1960). Thus, whilst being closely allied to cases of reversion in which the apex itself reverts, vegetative proliferation was not suitable for the study intended here.

Reversions in which the apical meristem itself resumes vegetative growth after a period of flowering can be divided into two categories: inflorescence reversion and flower reversion. In inflorescence reversion the terminal meristem stops initiating bracts with flowers in their axils, and may initiate sterile bracts and then leaves, as occurs naturally in Ananas comosus (Kerns, Collins and Kim, 1936). Alternatively, after initiating a zone of leafy bracts with aborted or proliferous flowers in their axils, the terminal meristem may resume initiation of leaves with vegetative branches in their axils, as for example in Sinapis alba (Bernier and Dath, 1962; Bagnard, Bernier and Arnal, 1972; Bagnard, 1980a and b, 1983) and Perilla (both green- and red-leaved)<sup>+</sup> (Jacobs and Raghavan, 1962; Zeevaart, 1969). Since many plants, both photoperiodically sensitive and day neutral, can, under appropriate conditions, be caused to undergo inflorescence reversion (see Appendix 1), the implication is that the floral state is not necessarily a permanent one. However, the same problem is associated with study of reversion in these plants as is associated with study of their evocation. This is that the changes taking place

<sup>+</sup> The taxonomy of Perilla spp. is confused (see Zeevaart, 1969); for clarity I have quoted leaf-colour throughout.



in the meristem at the transition to and from flowering are concerned with modifying the pattern in which leafy parts, with either flowers or vegetative buds in their axils, are produced by the apex, rather than with the initiation of flower parts. Therefore, plants showing inflorescence reversion were not examined as possible experimental systems for the research to be described.

Floral reversion is the situation in which the shoot meristem returns to leaf initiation after having begun to flower. In only three species has detailed study of the conditions bringing about floral reversion been made. The results of this work will now be briefly summarized.

In the LDP Anagallis arvensis L., ssp. phoenicea Scop. a single long day of 24 h induced flowering at the axillary meristem of a single node of the plants (Fontaine, 1972b). Floral reversions occurred at these meristems in response to this treatment, their frequency varying according to the plastochron stage of the terminal meristem at the time of induction. This variation occurred because the age of the axillary meristems on arrival of the floral stimulus was a critical factor in determining whether vegetative branches, normal flowers, or floral reversions were produced during their subsequent development (Fontaine, 1972a and b). Previously, the reversions observed had been thought to result from a change in the balance between vegetative and floral stimuli during the development of the flowers involved (Chouard, 1957; Brulfert and Chouard, 1961; Brulfert, 1961, 1965; Brulfert and Fontaine, 1967). More



recent work has also been interpreted in terms of the latter hypothesis (Fontaine, Lacombe and Brulfert, 1973; Brulfert, Imhoff and Fontaine, 1976; Fontaine, Dugué and Miginiac, 1977).

In the SDP Pharbitis nil Chois. strain 'Violet', floral reversions have been reported to occur at particular axillary meristems, in this case as a result of high temperature during the period following the single inductive long night (King and Evans, 1969; King, 1983). Only the axillary meristems at nodes 5 and 6 reverted, whilst the lower axillary meristems flowered normally, suggesting that sensitivity to the high temperature treatment may be related to the stage of meristem development as in Anagallis. Occasional reversion of the terminal meristem and the axillary meristems at nodes 2 and 3 followed  $\gamma$ -irradiation of induced plants of Pharbitis, again suggesting that the occurrence of reversion depended on the developmental stage of the meristems at the time of treatment (Wada, 1968).

The SDP Impatiens balsamina (cv. Buisson Fleuri) showed floral reversion at both the terminal and axillary meristems when the plants were transferred to long days at any time during flower formation (Debraux and Simon, 1967, 1969a and b; Simon, 1973, 1974). The variety Rose, which formed a terminal flower only when all the axillary buds were removed as they appeared (Nanda and Purohit, 1967), showed reversion of the axillary meristems on transfer from short to long days during flower



development (Nanda and Krishnamoorthy, 1967; Krishnamoorthy and Nanda, 1967, 1968). In the variety Rose axillary flowering was also induced by gibberellic acid, and reversion occurred if applications of this substance were not repeated regularly during flower development (Nanda, Krishnamoorthy, Anuradha and Lal, 1967).

It is clear from the work described on Anagallis, Pharbitis and Impatiens that floral reversion has been recognised to be of interest in the context of normal flower formation. In Anagallis and Pharbitis it has been shown that the stage of development of the meristems at the time of treatments designed to bring about reversion is important. Furthermore, in each of the three species different treatments have been used to bring about reversion: a minimal inductive treatment (1 long day) in Anagallis; high temperature or  $\gamma$ -irradiation of induced plants in Pharbitis; and transfer from short days to long days in Impatiens. However, plants showing floral reversion have not been used to study evocation; nor has detailed study of the growth and development of reverting meristems been carried out. This is probably because floral reversion usually occurs in only a small percentage of the plants treated, and is often unpredictable in its precise time of occurrence in relation to the factor(s) believed to bring it about. The position of the abnormal flowers on the plants, and the final form of these flowers, is also often unpredictable (Brulfert, 1965; Wada, 1968).

These difficulties have therefore not allowed an experimental approach to the study of reversion and its bearing on evocation, in the past. For this to be possible



reversion must be sufficiently consistent and predictable in all or most of the plants. To produce a suitable experimental system a preliminary study was made on Anagallis, Pharbitis and Impatiens. Of these, only Impatiens could be made to revert from flowering in a sufficiently predictable manner to allow experimentation.

The variety used in the studies to be described was Impatiens balsamina L. (cv. Dwarf Bush Flowered). This variety forms zygomorphic lateral flowers and a large actinomorphic terminal flower with many floral parts. It was easiest to study flowering and reversion at this terminal meristem. Initially experimental regimes were developed for the induction of flowering and for reversion in this terminal flower. These are described in Chapter 3 (pp.53-68). Then the changes in apical growth rates and in the arrangement, size and rate of initiation of primordia were compared in flowering and reverting plants. The aim was to establish whether such changes, which are usually associated with flowering (Bernier et al., 1981b) are necessarily linked to the production of flower parts in Impatiens. If they are, they would presumably be reversed on reversion, and would therefore warrant particular attention as possible essential, causal, events for the initiation of flower parts. The results are presented in Chapter 4 (pp.69-118).

The response of the reverted apex to further induction was then investigated, to determine whether the reverted apex was partially evoked. The changes in apical growth rates and in the arrangement, size and rate of initiation



of primordia were compared in reverted and re-flowering plants. Any changes that are essential to flower part initiation would be expected to occur on re-flowering, as they occurred on flowering. This work also aimed to establish whether the reactivity of the reverted apex to SD was the same as that of the vegetative apex, and, if not, to find out the ways in which the flowers formed by the two types of apices differed. The results are presented in Chapter 5 (pp. 119-64)

The final section of work, described in Chapter 6  
165-  
(pp./ 226) involved study of the development of primordia destined, as a result of reversion, to become parts intermediate between leaves and petals. It was asked whether these parts had their mature form determined at the time of their initiation, or whether their intermediate character resulted from a switch from the petal to the leaf developmental pathway after initiation. The aim was to establish if the primordia themselves responded to stimuli resulting from alterations in photoperiod, and, if they did, whether the characteristic features of mature reverted plants could be explained in terms of this response. Following from this, a study was made of the time at which parts on the reverted meristem became committed to leaf development. It was observed that commitment occurred at different times in different parts of the primordium. Ultrastructural changes were therefore followed in cells from different parts of the primordium, to establish whether earlier differentiation (vacuolation, plastid replication) was correlated with earlier commitment to leaf development.



CHAPTER 2

MATERIALS AND METHODS



## CHAPTER 2: MATERIALS AND METHODS

### 2.1 Plant Material

Mixed seed of Impatiens balsamina L. (cv. Dwarf Bush Flowered) was obtained from Wm. K. McNair, Portobello, Edinburgh, U.K.

### 2.2 Growth Conditions

The plants were grown in controlled environment rooms. Long days (LD) were of 24h, consisting of 8h light, provided by Philips 65-80W white fluorescent tubes supplemented by tungsten bulbs (total photosynthetic photon flux density 275-285  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  at the top of the plants on day 0; see section 2.4), followed by 16h light of low photosynthetic photon flux density (6  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  at the top of the plants on day 0) provided by tungsten bulbs. Temperature was  $22 \pm 1^{\circ}\text{C}$ , and relative humidity 55-65 per cent.

Short days (SD) were the same as LD, except that the 8h light period was followed by 16h dark, and temperature was  $21 \pm 1^{\circ}\text{C}$ .

### 2.3 Seed germination, growth of plants and selection for developmental uniformity

Preliminary experiments suggested that variable seed germination rate was responsible for much of the variation in seedling emergence rate (Section 3.1, p.54). Therefore, after imbibition of seeds on moist filter paper in Petri dishes in LD for 65h, only seeds with emerged radicles 3-4 mm long were sown. These seeds were sown at a depth of 1cm in moist 3:1 peat:sand mix (G.C.R.I. No. 1



Formulation - see Appendix 2). Depending on the sampling regime, and duration, of the experiment, 24 to 35 seeds were sown per 37 x 23 x 6 cm holed tray, and watered until the soil was thoroughly wet. The trays were then placed in LD. The soil was kept moist by application of 200 ml distilled water per tray every day, except every third day when 200 ml full-strength Hoagland's solution (Hoagland and Arnon, 1950; see Appendix 2) was supplied.

Seven days after sowing the young seedlings were selected for developmental uniformity; all those in which the first leaf was not 4-8 mm long were discarded.

#### 2.4 Treatments causing flowering and reversion

The 7th day after sowing, when seedlings were selected for developmental uniformity, marked the beginning of treatments in inductive SD, and was designated day 0. After selection a sample of five or six plants was taken, to establish the total number of leaves plus primordia present. Plants to be induced to flower were transferred to SD, where they remained until dissection 6-7 weeks later, when the terminal flower had fully opened. Reversion was brought about by transfer back to LD after varying numbers of SD; the plants were dissected at the same time as the plants given continuous SD. Re-flowering of reverted plants was brought about by transfer back to SD of plants given 5SD plus varying numbers of LD. These plants were dissected when the terminal flower had fully opened. Vegetative controls were plants that remained in LD from day 0. In all treatments about  $\frac{1}{8}$ th of the plants flowered only at the axillary meristems, and in



LD a further  $\frac{1}{8}$ th of the plants showed an anomalous form of terminal flower formation. Since both these types of plants were easily distinguished from other plants by their modified phyllotaxis and/or their precocious axillary buds, they were excluded from the analyses described here.

## 2.5 Selection for uniformity of reversion response

Early experiments indicated that the reversion response of red-flowered plants was more uniform than that of plants with other flower colours (see Table 9, p. 67). Therefore, in subsequent experiments, analyses were confined to plants with red flowers in the following way. White-flowered plants always had green stems. These could therefore be excluded at an early stage. Plants with flowers of other colours all had red stems, and so could not be distinguished on this basis. Therefore, during the experimental treatments only the terminal shoot of these plants was sampled and prepared for analysis; the decapitated plants were then allowed to grow on, so that the axillary meristems grew out. After transfer to SD (where necessary) these plants flowered. Only those samples which came from red-flowered plants were used in the experimental analyses. In the experiments where this sampling procedure was used only red-flowered (control) plants were scored for their flowering and reversion response at maturity.

## 2.6 Growth of the apex and primordia

### 2.6.1 Number of leaves plus primordia

At each sampling time not less than five plants were



dissected under a dissecting microscope, and the total number of leaves plus primordia (excluding the cotyledons) counted on each plant. Values of total number of leaves plus primordia obtained during sampling of plants for the analyses described below were also included. Rates of primordium initiation per day were calculated as described in the Results section (p. 74).

#### 2.6.2. Developmental stages

Plants were grouped into developmental stages according to the total number of leaves plus primordia present at the time of sampling, in all the growth analyses to be described. These stages (see Tables 15-17, 30-31, <sup>pp.</sup> 87, 93, 95 and 147, 150 ) were chosen to correspond to distinct phases of development in all treatments, and allowed comparison between the different treatments, and different phases of development during the same treatment.

#### 2.6.3 Distances of primordia below the apical surface

The shoot apex of Impatiens has the shape of a flat-topped cone (see Fig. 15, p. 89 ); the flat top bears the young primordia and will be referred to as the apical surface. The distances of primordia below the apical surface were measured to provide an estimate of the vertical relative growth rate per plastochron of the apex, and of the length of the stem frustum on initiation. This frustum includes the stem tissue adjacent to the primordium, and the stem tissue between the abaxial insertion point of the primordium and the adaxial insertion point of the next oldest



primordium (Fig. 1). These tissues give rise to the node and internode, respectively, in the mature plant, the internode being that below the leaf in question (i.e. for leaf 1 the internode is the epicotyl). To provide these measurements between three and 20 plants were taken at various times after the beginning of each experiment, the unfolded leaves and the cotyledons were removed, and the remaining shoot plus primordia fixed in formalin:acetic acid: 50 per cent ethanol (1:1:18 by vol.) for at least 24h. The fixed tissues were dehydrated through an ethanol series and embedded in wax. Serial transverse sections, 5  $\mu$ m thick, were stained with 0.5 per cent Toluidine Blue O (BDH Chemicals Ltd., Poole, Dorset) (O'Brien and McCully, 1981) or by a modification of the method of Sharman (1943). The latter method is illustrated in Fig. 2. From these sections the developmental stage of each plant was determined by counting the number of primordia in the first transverse section to graze the surface of the shoot apex, and adding to this the unfolded leaves removed before embedding, to give the total number of leaves plus primordia.

The distances of the successive leaves or primordia below the apical surface were then measured by recording, for each, the section in which connection between its base and the stem was half complete. Where the succession was in doubt (e.g. in whorls or pseudo-whorls), it was assumed that the primordia were initiated with successive angular separations of approximately  $137^{\circ}$ .

In some cases (particularly in flowering or reverting



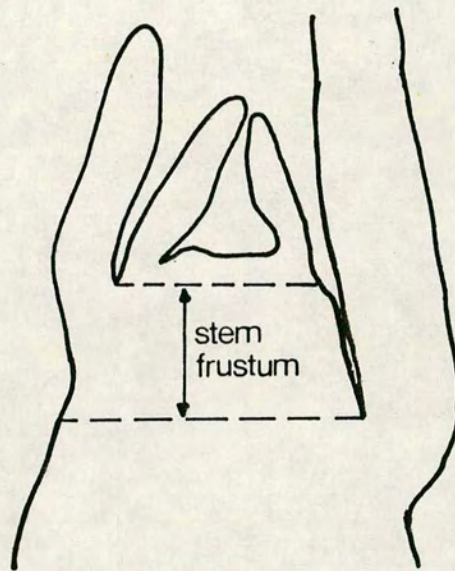


Figure 1. Illustration of the stem frustum: outline drawing of a longitudinal section of the shoot apex of Impatiens.



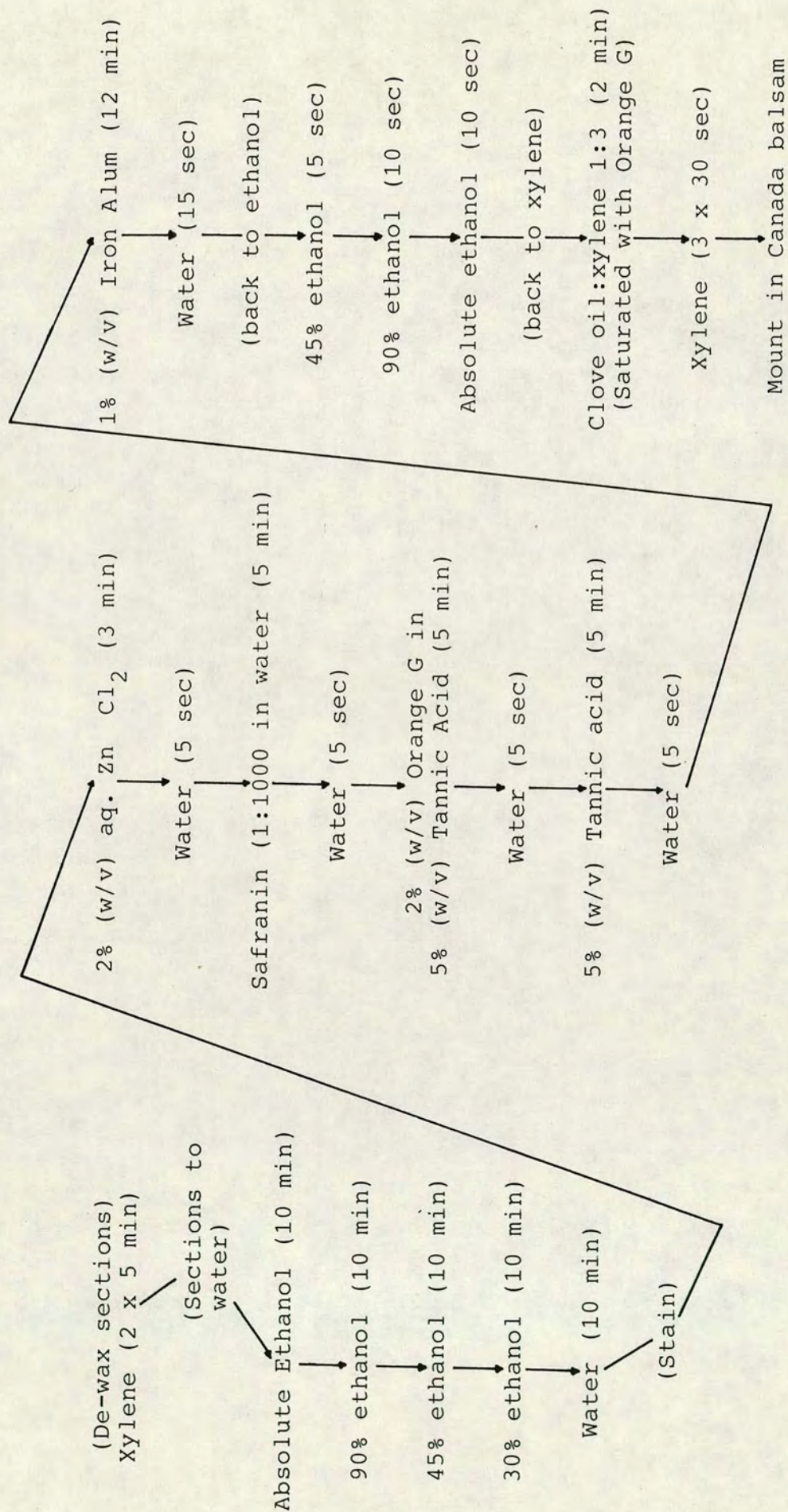


Figure 2. The modification of Sharman's (1943) method used for staining transverse and longitudinal sections of the shoot apex.



plants) shoot growth was not straight, resulting inevitably in some skewed sections. In these cases the distances of the primordia below the apical surface were measured in relation to the youngest primordium on the same side of the apex.

The number of primordia on the apical surface was estimated from these measurements. Primordia with a mean transformed distance,  $\log_e(\underline{x}\mu\text{m} + 1)$ , of less than  $\log_e(5\mu\text{m} + 1)$  below the apical surface were defined as being on the apical surface (see also Fig. 17, p. 97).

#### 2.6.4. The plastochron ratio

The plastochron ratio ( $\underline{r}$ ) is the ratio between the transverse distance of the primordium from the apical centre relative to that of the next primordium to be produced (Richards, 1951). This was measured:

- a) using the serial transverse sections prepared as described in Section 2.6.3. In this case a camera lucida drawing was made, or a photograph was taken using a Zeiss photomicroscope and printed at known magnification, of the first transverse section to graze the apical surface.
- b) on photographs of the shoot apex, taken with a Cambridge Stereoscan 250 Scanning Electron Microscope (SEM). In this case six plants were taken on days 8, 10 and 12 following selection, the cotyledons and the nine oldest leaves were removed, and the remaining shoot plus young parts was frozen in liquid nitrogen. These stem tips were



each placed in a screw-topped tube containing a small amount of drying agent (molecular sieve type 4A; BDH) and a large excess of absolute methanol, at  $-80^{\circ}\text{C}$ . They were then freeze-substituted in absolute methanol for 2 wk at  $-80^{\circ}\text{C}$ , 1 wk at  $-40^{\circ}\text{C}$ , 1 d at  $-20^{\circ}\text{C}$ , 15 h at  $4^{\circ}\text{C}$  and 2 h at room temperature (method modified from Robards, 1978). After transfer to fresh absolute methanol the tissues were passed through a methanol-acetone series to absolute acetone. Following critical point drying with carbon dioxide the apices were mounted on stubs, and the older primordia were carefully removed, to reveal the apical surface, whilst leaving clear outlines of the positions of the primordium bases around the apex. The dissected apices were gold-coated and viewed from directly above in the SEM at an accelerating voltage of 40 kV and a spot size of 6 or 7. Photographs were taken at successive magnifications, so as to include all the young primordia and the bases of those removed during dissection.

The outlines of the primordia were traced from the photographs or camera lucida drawings, and the centre of each was estimated using circles of appropriate size and equalizing parts of the primordium bases outside the circles.

The plastochron ratio was measured most easily by the method of Maksymowych and Erickson (1977), in which only the order of initiation of primordia need be known.



In vegetative plants, and for those primordia on flowering and reverting meristems initiated before or during the transition to flowering, in which the order of initiation could be determined from the genetic spiral, this method was therefore used. The order of initiation of primordia became difficult to establish objectively after the transition to flowering, so the plastochron ratio was estimated from primordia initiated after this time in the following way. Firstly, the position of the apical centre was fixed from the intersection point of the radii from the older primordia whose order of initiation was known, and whose distances from the apical centre could be calculated by the method of Maksymowych and Erickson (1977). The distances from this centre of those primordia initiated after the transition to flowering were then measured. The order of their initiation was taken to be a function of their radial distances, and the plastochron ratios were calculated by the method of Richards (1951).

During the later stages of flowering (Stamen/carpel stage), and after reversion had taken place, the centre of the apex could no longer be fixed as described above, because of possible shifts in the relative positions of older primordia as they were displaced away from the apex. In these cases the position of the apical centre was fixed by equalizing the radial distances of the primordia of the youngest whorl or pseudo-whorl as far as possible. The plastochron ratios were then calculated by the method of Richards (1951).

The areas of primordia on initiation, relative to the area of the bare apical surface, and the area relative growth rates per plastochron of the apex ( $2\log_e r$ ; Richards, 1951), were calculated from the plastochron ratios derived



from primordia nearer the centre than, and including, the first primordium below the apical surface.

Areas of the primordium bases and the free apical surface were also measured from SEM photographs, using a Reichert-Jung 'Videoplan' digitiser.

#### 2.6.5. Area and vertical relative growth rates per day of the apex

These were obtained as area and vertical relative growth rates per <sup>6</sup>platochron respectively, multiplied by the rate of initiation of primordia per day.

#### 2.6.6. Apical shape

Plant material was prepared, embedded, and stained, as described in Section 2.6.3., but was sectioned longitudinally at 10 $\mu$ m intervals. Photographs were taken of the section in which the youngest primordium was visible, to illustrate apical shape.

#### 2.6.7 Lengths and widths of primordia

In an allometric study (see Section 6.1, p.166) lengths and widths of primordia between about 0.1 and 10.0 mm long were measured after dissecting out the apex under the dissecting microscope. The primordia were removed at their points of insertion on the stem, laid flat on the stage of the dissecting microscope, and their outlines drawn using a camera lucida. Subsequently, the length from the middle point of the primordium base to the most distal point of the primordium was measured on these drawings. Width was then



measured halfway along this length; basal width was measured across the base of the primordium, and the surface area of the primordium measured on the drawings using a Reichert-Jung 'Videoplan' digitiser.

In other studies primordium length was measured on the serial transverse sections prepared as described in Section 2.6.3 (p. 20). The number of 5  $\mu\text{m}$  sections between the section in which the tip of the primordium appeared and that in which connection between its base and the stem was half complete was counted, and primordium length obtained as: number of sections  $\times$  5 ( $\mu\text{m}$ ).

In the stereological analysis primordium length was measured from longitudinal sections of resin-embedded material as described in Section 2.7.3 (p.33 ).

#### 2.6.8 Areas of mature parts occupied by petal pigment

To quantify the effects of different photoperiodic treatments on flower form, the percentage of the surface of the mature parts of the flower, or reverted flower, occupied by petal pigment, was measured as follows. The plant parts were removed from the plant at their points of attachment to the stem, and laid flat on a white surface. Their outlines, and the areas over which coloured (petal) pigment occurred, were traced. These outlines were placed on a digitising tablet, traced with a magnetic cursor, and the areas with and without coloured pigment were calculated by a Reichert-Jung 'Videoplan' digitiser. The accuracy of area measurements made by the digitiser is greater than 99 per cent. The outlines were photocopied to



provide a permanent record.

To ensure that the parts of all flowers, regardless of treatment, were measured at the same morphological stage, dissection and measurement was carried out on fully-opened flowers. A fully-opened flower was defined as one in which the stamens were clearly visible at the centre of the corolla. In plants given SD from day 0 this stage was reached 6-7 weeks after day 0. Reverted plants (5SD + LD) were also measured at this time. In re-flowering plants (5SD + xLD + SD), the terminal flower often took slightly longer to open fully, so dissections were usually carried out about 7-8 weeks after day 0. Checks were made to ensure that by about six weeks after day 0 the percentage of the surface occupied by coloured pigment had become constant, by photographing living specimens, and then re-photographing them about 10 days later. Measurements made from tracings of these photographs showed that during the 10 day period the percentage of the surface of these parts occupied by petal pigment had not changed appreciably.

## 2.7 Stereological analysis of developing primordia

### 2.7.1 Tissue preparation

The aim of the analysis was to examine ultrastructural changes during primordium development, in parts 32-34 (where part 1 = the oldest leaf, excluding the cotyledons) of reverted plants. Samples were therefore taken at various times during the experimental treatment, and parts 1 to 31 removed under a dissecting microscope. The remaining shoot tips were quickly transferred to 3 per cent glutaraldehyde



(E.M. grade; Agar Aids Ltd, Stansted, Essex) in 0.05 M phosphate buffer of pH 7.2. After fixation for 2-12 h, the shoot tips were washed three times, for 20 min each time, in phosphate buffer, and then rinsed for a further 20 min in distilled water.

The shoot tips were then immersed in 2 per cent osmium tetroxide (aq., Agar Aids) for 1 h, after which time they were rinsed 3 times in phosphate buffer, again for 20 min each time. They were dehydrated in an alcohol series, passed through an alcohol:propylene oxide series to absolute propylene oxide and finally to 'Araldite' (as recommended by Pickett-Heaps; see O'Brien and McCully, 1981) via a propylene oxide:'Araldite' series. The series used are shown in Table 3.

After leaving the shoot tips for 12 h in absolute 'Araldite' in an unstoppered vial, each was removed in turn. Excess 'Araldite' was dabbed off gently with a paper towel, and each was embedded in fresh 'Araldite'. Each shoot tip was orientated with its stem axis parallel to the short side of the embedding well, so that sectioning the block face would give longitudinal sections. The embedded shoot tips were left for 48 h at 60°C whilst the 'Araldite' cured.

#### 2.7.2 Sectioning and staining of primordia

After trimming the block face to the minimum area which included all the primordia and the apex itself, sections were cut on a Reichert-Jung 'Ultracut' ultra-microtome. All sections were cut on to water with glass



TABLE 3. Alcohol to araldite series used in preparing material for resin embedding

Proportions of solvent			Time (h)
Distilled water : Absolute ethanol			
1	:	0	1
9	:	1	2
8	:	2	2
6	:	4	Overnight
4	:	6	1
2	:	8	1
1	:	9	2
0	:	1	1
0	:	1	$\frac{1}{2}$
Propylene oxide : Absolute ethanol			
0	:	1	$\frac{1}{2}$
1	:	3	1/4
1	:	2	1/4
1	:	1	1/4
2	:	1	1/4
3	:	1	1/4
1	:	0	1/4
Propylene oxide : Absolute 'Araldite'*			
3	:	1	1
2	:	1	2
1	:	1	12
1	:	2	12
1	:	3	12
0	:	1	12

\* Made up from the following components:

Araldite CY212	178.4 g
Dodecenyl succinic anhydride	148.8 g
Dibutyl phthalate	3.2 g

To this stock 'Araldite' 1 part to 40 of Benzyldi-methylamine (hardener) was added a maximum of 24 h before use.



knives made on an LKB Knifemaker (Type 7801A). The sections were stretched by passing chloroform vapour over them. 1  $\mu$ m thick sections, for light microscopy (LM), were transferred to a drop of water on a slide using a platinum wire loop. The slide was then placed on a hot plate at 60°C to stretch and dry the sections. 70-80 nm sections (as determined by interference colours) for Transmission Electron Microscopy (TEM) were removed from the water surface by placing a 200-mesh grid, or a 1 mm slot grid (Agar Aids) under the water and lifting it up gently below the sections. Before use all grids were coated with Formvar (Agar Aids) by floating them on the surface of a 0.5 % (W/V) solution of Formvar in chloroform (Roland, 1978).

The following procedure was devised to obtain sections from equivalent parts of different primordia, for both LM and TEM. At the beginning of sectioning of each block, sections 1-2  $\mu$ m thick were cut until the outer whorl of primordia was reached. Then, for the first primordium reached in the outer whorl, profiles were observed in the light microscope at intervals of not more than about 30  $\mu$ m maximum. When the primordium profile remained approximately the same size in successive sections, the following three steps were carried out.

- 1) four successive 1  $\mu$ m sections were cut, transferred to a slide, stained (see below), and mounted. (Four sections were cut to ensure that a least one good section was available for photography.)
- 2) A maximum of 10 successive 70-80 nm sections were



cut and mounted on Formvar-coated grids. Usually two grids were prepared carrying these sections.

- 3) five successive 2  $\mu\text{m}$  sections were cut and discarded.

This series of steps was repeated three times, so that for each sectioned primordium three sample sections, near the midline of the primordium, but about 10 to 14  $\mu\text{m}$  <sup>for</sup> apart, were obtained/both LM and TEM. Further sections were then made across the apex, repeating the above procedure for each primordium larger than that initially sectioned. When the apex had been completely sectioned, the slides (for LM) and the grids (for TEM) bearing sections from the three largest primordia were retained and used for further analysis. These three primordia were taken to be parts 32, 33 and 34, numbering from the base of the plant upwards.

All 1  $\mu\text{m}$  sections were stained in a drop of 0.5 per cent (W/V) Toluidine Blue 0 (BDH) in 1 per cent (W/V) borax (BDH) for about 5 mins at 60°C on a hotplate. After rinsing with 70 per cent ethanol and drying on the hotplate these sections were mounted in 'Araldite'. Sections for TEM were stained with 2 per cent (W/V) uranyl acetate (Analar; BDH) and lead citrate (Analar; BDH) as described by Roland (1978). They stained most effectively if left on 2 per cent (W/V) uranyl acetate for 1 h, and then on lead citrate for 15 min.

### 2.7.3 Photography of the sectioned primordia; location of areas for stereological analysis

#### A. Light microscope photographs

For each of the three primordia sampled from each apex,



at least one 1  $\mu\text{m}$  thick section was available for photomicroscopy in the / at each of three sites separated from one another by about 10 to 14  $\mu\text{m}$ . These sites were located around the midline of the primordium. A section from each of these sites was photographed using a Zeiss photomicroscope and Technical Pan film (Eastman Kodak Co., Rochester, New York). The film was developed for moderate contrast in HC110 developer (Kodak). Prints were made at a standard enlargement, giving a final magnification of 93 X. The length of the primordium was measured on each of these prints as illustrated in Fig. 3. This procedure gave three length measurements for each primordium; the longest (usually that from the middle of the three sites) was taken as the best estimate of primordium length, and recorded. The prints were then used to locate four areas of the primordium designated a, b, c and d, to be analysed stereologically using TEM. Areas a and b were 1/4 of the way down the primordium, and areas c and d were 3/4 of the way down the primordium. Each area consisted of the two cell layers immediately beneath the epidermis at these points; area a and c were on the inner, adaxial side of the primordium, and areas b and d were on its outer, abaxial side (see Fig. 3).

#### B. Transmission electron microscope photographs

Areas a to d were located on each selected primordium on ultra thin sections viewed in a Jeol 100S transmission electron microscope at an accelerating voltage of 60-80 kV, using as a guide the light microscope photograph of the



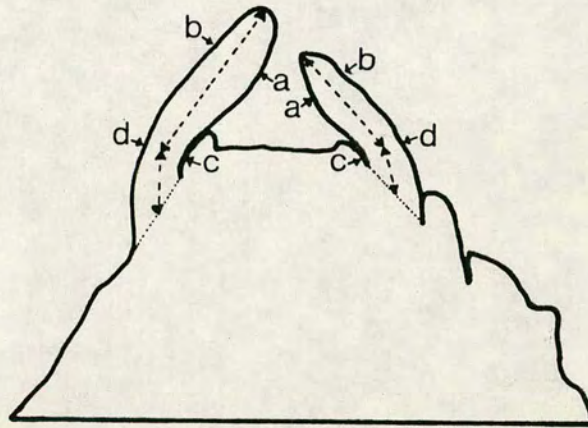


Figure 3. Outline drawing of a longitudinal section of the shoot apex of Impatiens illustrating the method used to measure the length of a primordium, and the location of areas a to d used for stereological analysis. Dotted lines with arrows indicate axes along which the length of the primordium was measured.



adjacent thick section. Photographs were then taken of each area at magnifications of 1000 X and 5000 X on the plate. These magnifications were selected so that both gross cellular changes (level 1 analysis, 1000 X magnification) and changes in the number and volume of plastids (level 2 analysis, 5000 X magnification) could be followed, whilst ensuring adequate replication at each level. The specimens were photographed on Ilford technical film (Ilford Ltd., Basildon, Essex) or Kodak electron image film (Kodak) (6.5 x 9 cm). The former was developed in Ilford 'P.Q. Universal' developer (10 per cent V/V in water containing 0.4 per cent Ilford 'Ilfotol' wetting agent) for 4 min at 20°C, and the latter in Kodak 'D-19' developer (33 per cent V/V in water) for 4 min at 20°C.

For each of the four areas (a to d) a photograph was first taken at a magnification of 1000 X so as to include the two cell layers under the epidermis. Then the grid was moved until these two cell layers fell approximately across the centre of the photofield. The magnification was increased to 5000 X, and, after ensuring that no epidermal cells were in the photofield, a photograph was taken. This method ensured that within the two cell layers in the areas to be analysed, the 5000 X photograph was taken at random. Occasionally the field of view at this magnification included parts of cells from the third cell layer below the epidermis.

#### 2.7.4 Analysis of the Photographs

##### Level 1

Photographs were taken at a magnification of 1000 X



and enlarged to give a linear magnification of 2,850 X on the prints. A Weibel pattern multi-purpose test grid (Weibel and Bolender, 1973) was superimposed on each photograph. The grid contained 168 points (pairs of points being joined by a grid line), and was of dimensions  $12z \times 12.12z$ , where  $z = 10 \text{ mm}$ . The total grid line length was equivalent to  $294.0 \text{ } \mu\text{m}$ , and the total grid area to  $1781.6 \text{ } \mu\text{m}^2$ , at the standard magnification. The grid was placed over the photograph so that the longer sides of the grid rectangle ran approximately parallel to the side of the primordium, and the side of the grid nearest the outer edge of the primordium touched the inner walls of the epidermis (see Fig. 4). The total number of points falling on the two cell layers below the epidermis was then recorded. The total line length covering these cells was estimated as:

$$\text{total line length} = \frac{x}{168} \times 294 \text{ } (\mu\text{m}),$$

and the total area counted was estimated as:

$$\text{total area} = \frac{x}{168} \times 1781.6 \text{ } (\mu\text{m}^2).$$

In both cases  $x$  is the number of points falling on the two cell layers. The number of these points falling on nuclei, vacuoles, cytoplasm, and cell walls or intercellular space, was recorded, along with the number of cell profiles with more than half their area within the grid boundary. The length and width of each of these cell profiles was also measured and recorded.



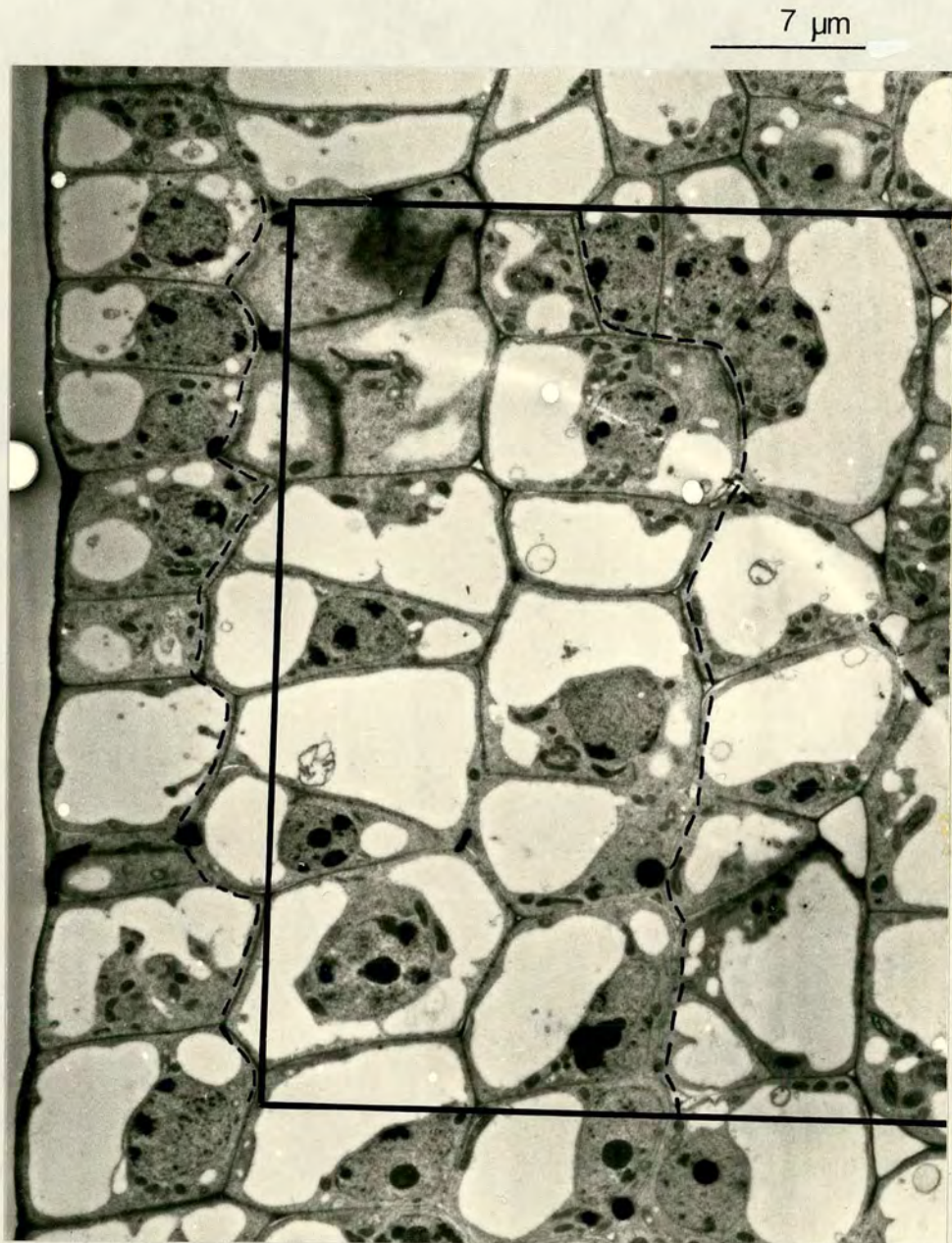


Figure 4. Position of the grid in the level one stereological analysis. The analysis was confined to the two cell layers below the epidermis. Area c is the area of the primordium illustrated in this photograph. Solid lines indicate the sides of the grid; broken lines indicate the boundaries of the two cell layers measured.



## Level 2

The aim of the analysis at this level was to determine the number of plastids per cell, and the volume of these plastids, in primordia at different stages of development. Photographs were taken at a magnification of 5000 X, and enlarged to give a linear magnification of 18500 X on the prints. A Weibel pattern multi-purpose test grid was superimposed on each photograph. The grid contained 100 points (pairs of points being joined by a grid line), and was of dimensions  $10z \times 8.66z$ , where  $z = 20$  mm. The number of points falling on cytoplasm, and plastids, was recorded along with the number of plastid profiles with more than half their area within the grid boundary. The length and width of each of these plastid profiles was also measured and recorded.

### 2.7.5 Analysis of the data

The procedure described gave a maximum of three sets of point counts for each area (a to d) in each primordium sampled, at both levels 1 and 2. These sets of point counts were pooled so that for each area of each primordium a single set of point counts was available for calculation of the required values. Pooling the data in this way meant that each estimate was derived from between about 50 and 200 point counts. The following calculations were then carried out.

## Level 1

The number of cells per unit area of tissue was calculated as



$$N_{A \text{ cells}} = \frac{Q_{A \text{ cells}}}{A_{T \text{ tissue}}}$$

where  $Q_{A \text{ cells}}$  is the number of cell profiles with more than half their area within the grid boundary, and  $A_{T \text{ tissue}}$  is the area of tissue counted. The fraction of the tissue volume occupied by cells was

$$V_{V \text{ cells}} = \frac{\text{Number of points falling on cells}}{\text{Number of points falling on tissue}}$$

The number of cells per unit volume of tissue was then calculated as

$$N_{V \text{ cells}} = \frac{N_{A \text{ cells}}^{\frac{3}{2}}}{V_{V \text{ cells}}^{\frac{1}{2}}} \cdot \frac{K}{\beta}$$

(Weibel and Bolender, 1973; Steer, 1981), where  $\beta$  is the coefficient of configuration.  $\beta$  was obtained by calculating the mean length:width ratio of the counted cells, and reading  $\beta$  from the graph given in Weibel and Bolender (1973), making the assumption that the cells were cylinders. To check that during the course of primordium development  $\beta$  did not change appreciably, values were first obtained for each area (a to d) from primordia divided into classes according to their length. Since no consistent variation was obtained ( $\beta$  varying from about 1.75 to 1.95), a mean value was obtained for each area (a to d). This varied between 1.83 and 1.87, and was derived from at least 350 measurements in each case.  $K$  was put equal to 1.0, making the assumption that the cells were all of the same size

\* Symbols are those adopted by Weibel & Bolender (1973), Steer (1981), and recommended by the International Society for Stereology.



(see Weibel and Bolender, 1973; Steer, 1981).

Average cell volume was calculated as

$$V_{\text{cells}} = \frac{V_V \text{ cells}}{N_V \text{ cells}} \quad (\mu\text{m}^3)$$

This value of  $V_{\text{cells}}$  was calculated for each area (a to d) in each primordium sampled. Then, again for each area in each primordium sampled, the percentage of the cell occupied by cytoplasm, nucleus and vacuole was calculated as

$$V_{Vi} = \frac{\text{Number of points falling on component } i}{\text{Number of points falling on cells}} \times 100 \quad (\%)$$

where component  $i$  was cytoplasm, nucleus or vacuole. The volume of each component per cell was then calculated as

$$V_{i.\text{cell}}^{-1} = \frac{V_{Vi}}{100} \times V_{\text{cells}} \quad (\mu\text{m}^3)$$

## Level 2

The area of cytoplasm per area counted was calculated as

$$A_T \text{ Cytoplasm} = \frac{\text{Number of points falling on cytoplasm}}{\text{Total number of points counted}} \times \text{Area of tissue counted} \quad (\mu\text{m}^2)$$

and the number of plastids per unit area of cytoplasm calculated as



Number of plastid profiles with more than half their area within the grid boundary

$$N_A \text{ plastids} = \frac{\text{Number of plastid profiles with more than half their area within the grid boundary}}{A_T \text{ cytoplasm}}$$

The fraction of the cytoplasmic volume occupied by plastids was

$$V_V \text{ plastids} = \frac{\text{Number of points falling on plastids}}{\text{Number of points falling on cytoplasm}}$$

The number of plastids per unit volume of cytoplasm was then calculated as

$$N_V \text{ plastids} = \frac{N_A^{\frac{3}{2}} \text{ plastids}}{V_V^{\frac{1}{2}} \text{ plastids}} \cdot \frac{K}{\beta}$$

(Weibel and Bolender, 1973; Steer, 1981), where  $\beta$  is the coefficient of configuration.  $\beta$  was obtained by calculating the mean length:width ratio of the counted plastids, and reading  $\beta$  from the graph given in Weibel and Bolender (1973), making the assumption that the plastids were ellipsoids.  $\beta$  values were obtained for each area (a to d) from primordia divided into classes according to their length. Unlike the  $\beta$  value obtained for cells (see p.40),  $\beta$  for plastids did appear to alter slightly during development, generally increasing from about 1.6 to 1.8 as the primordia grew. Therefore for each area (a to d) the value for  $\beta$  obtained for the length class in which the sampled primordium fell was used in the calculation. These values were obtained from between about 10 and 100 measurements in each case. K was put equal to 1.0, making the assumption that the



plastids were all of the same size (see Weibel and Bolender, 1973; Steer, 1981).

The number of plastids per cell was calculated as

$$N_{\text{plastids}} \cdot \text{cell}^{-1} = N_V \text{ plastids} \times V_{\text{cytoplasm}} \cdot \text{cell}^{-1};$$

$V_{\text{cytoplasm}} \cdot \text{cell}^{-1}$  was obtained for each area (a to d) in each primordium sampled in the level 1 analysis (p.41 ).

Average plastid volume was calculated as

$$V_{\text{plastids}} = \frac{V_V \text{ plastids} \times V_{\text{cytoplasm}} \cdot \text{cell}^{-1}}{N_{\text{plastids}} \cdot \text{cell}^{-1}} \quad (\mu\text{m}^3)$$

## 2.8 Gel electrophoresis

### 2.8.1 Extraction and precipitation of protein

#### Materials

Extraction buffer (EB) -

60 mM 'Trizma' base (Sigma London Chemical Co.,  
Poole, Dorset)

0.5 % (V/V)  $\beta$ -mercaptoethanol (Sigma)

pH 7.15

Sample buffer (SB) -

10 % (V/V) Glycerol

5 % (V/V)  $\beta$ -mercaptoethanol

2.35 % (W/V) Sodium lauryl sulphate (BDH)

625 mM 'Trizma' base, pH 6.8

Ammonium sulphate solution -

a saturated solution of enzyme grade ammonium sulphate (BDH)



Deoxycholate (DOC) -

0.15 % (W/V) sodium deoxycholate (Sigma)

Trichloroacetic acid (TCA)-

72 % (W/V) solution (BDH)

Molecular weight markers -

these were made up from the following proteins  
obtained from Sigma:

	Molecular Weight (Daltons)
Bovine Serum Albumin	68,000
Catalase	60,000
Aldolase	40,000
Carbonic Anhydrase	29,000
Soybean Trypsin Inhibitor	21,000
Myoglobin	17,200

Each of these proteins was present in the stock  
solution at a concentration of  $0.125 \text{ mg.ml}^{-1}$ .

#### A. Mature leaves and petals

Protein extracts of leaves or petals longer than  
1 cm were prepared as follows. Leaves were divided into  
stages according to length, and petals according to the  
stage of flower bud development, as shown in Table 4.

Each leaf extract was prepared from approximately  
1 g (fresh weight) of leaves (from main and axillary  
branches), excised at the base of the petiole. The leaves  
were ground, in 2 ml EB, in an ice-cold mortar and pestle.



TABLE 4. Mature Petal and Leaf stages devised for protein estimation and gel electrophoresis

Tissue	Stage	Length (cm)	Characteristics
Leaves	7	1-2	
	8	3-4	
	9	7-8	
Petals	6	0.6-0.8	Flower 1-1.2 cm long, unopened. No petal pigment
	7	0.8-1.0	Flower 1.4-1.6 cm long, unopened. No petal pigment
	8	1.0-1.6	Flower 1.6-2 cm long, petal colour just visible
	9	3.0	Flower fully open, petal colour fully developed



The extracts were transferred to 15 ml pre-cooled glass centrifuge tubes and centrifuged for 5 min at 1,250 g. 0.1 ml of each supernatant was then removed and used for protein estimation by the method of Lowry, Roseborough, Farr and Randall (1951). 1 ml of each supernatant was transferred to a 15 ml MSE centrifuge tube, and 3 ml of ammonium sulphate solution was added dropwise, with continuous mixing. The tubes were sealed with 'Parafilm' and stored at 4°C for 16 h whilst protein precipitation took place.

Extracts of petals at stage 6 were prepared from the terminal flowers of plants of the red-flowered genotype in the same way as described for leaves. Petals at stages 7-9 were detached at the base from the axillary flowers of red- and magenta-flowered genotypes and frozen immediately in liquid nitrogen. This allowed sufficient petals to be collected at each stage. The frozen petals were then extracted and their protein precipitated as described above.

After ammonium sulphate precipitation each extract was centrifuged at 4°C for 45 min at 10,000 g. The ammonium sulphate was poured off, the pellet was washed twice in 70 per cent acetone, mixed thoroughly during each wash and centrifuged for 1 h at 10,000 g (at 4°C) to re-pellet the precipitate after each wash. The 70 per cent acetone was then poured off, and the inside of the centrifuge tube dried carefully with a Kleenex 'Mediwipe'.

Between 50 and 200  $\mu$ l of SB was then added to each pellet, according to the amount of protein estimated to be



present. The absolute amount of protein in each sample was then calculated. The pellet was thoroughly mixed on a whirlimixer with the SB. Two samples from each extract were then prepared for gel electrophoresis. One sample contained 50  $\mu\text{g}$  total protein and the other 100  $\mu\text{g}$  total protein. Each sample was made up to 25  $\mu\text{l}$  with SB, and 2.5 $\mu\text{l}$  Bromophenol blue dye (BDH) was added. 10  $\mu\text{l}$  of molecular weight markers (see 'materials') was transferred to each of two Eppendorf tubes, and made up to 27.5  $\mu\text{l}$  with SB.

The tubes containing the samples and the molecular weight markers were placed for 3 mins in a boiling water bath; the samples were then transferred to the gel using a Hamilton microsyringe.

#### B. Leaf, petal and reverted part primordia

Extracts from these primordia (maximum size used = 4 mm) were prepared after first devising a series of size categories to allow sampling of primordia at different stages in the experiment. These categories are summarized in Table 5.

Because sufficient primordia could only be accumulated by repeated dissection of a large number of apices, the primordia were transferred immediately after dissection on to the frozen surface of 200  $\mu\text{l}$  of EB in Eppendorf tubes. The buffer was kept frozen by placing the Eppendorf tubes in solid carbon dioxide.

After the required number of primordia had been accumulated, the Eppendorf tubes containing the buffer and



TABLE 5. Primordium size ranges used for protein estimation and gel electrophoresis

Treatment				
<hr/>				
14SD	5SD+9LD	20SD	5SD+15LD	LD
<hr/>				
Size Range of primordia sampled (Parts 18-24) ( $\mu$ m)	150-1000	150-1000	1500-3000	500-1500
			1500-3000	1000-4000
<hr/>				
Number of primordia sampled	40	35	21	35
			21	19
<hr/>				



sample tissues were placed in a boiling water bath, and, after rapid thawing, the samples were ground with a clean glass rod. They were then re-frozen. This procedure was repeated three times; after the final freezing, the samples were again thawed rapidly and 50  $\mu$ l was removed and placed in a 15 ml glass centrifuge tube for protein estimation by a modification of the Lowry procedure described by Peterson (1977).

To the remaining 150  $\mu$ l of each extract 15  $\mu$ l of DOC was added, mixed thoroughly, and the mixture left for 10 mins at 20°C. 15  $\mu$ l of TCA was then added, mixed, and the mixture left to precipitate for 10 mins at 20°C. The samples were centrifuged for 12 mins at 12,000 g in a microcentrifuge ('Quickfit'), and then washed twice with 70 per cent acetone, and centrifuged for 6 mins at 12,000 g after each wash. After aspirating all the acetone from the pellets, 50  $\mu$ l of SB was added to each, and each sample was thoroughly mixed on a Whirlimixer. According to the results of the protein estimation, volumes of each sample solution sufficient to give 15  $\mu$ g of total protein were transferred to fresh Eppendorf tubes and made up to 50  $\mu$ l with SB. 2.5  $\mu$ l of Bromophenol blue dye (BDH) was then added to each sample.

2  $\mu$ l of molecular weight markers (see 'materials') was transferred to each of two further Eppendorf tubes, and made up to 52.5  $\mu$ l with SB.

The tubes containing the samples and the molecular weight markers were placed for 3 min in a boiling water bath. The samples were then transferred to the gel using



a Hamilton microsyringe.

## 2.8.2 Preparation, running and staining of gels

### Materials

Stock 30 % (W/V) acrylamide ('Electran', BDH)  
containing 0.8 % (W/V) bis-acrylamide (BDH)

Running gel buffer (made up at 4 X the required final  
concentration), pH 8.8:

1.5 M 'Trizma' base

0.4 % (W/V) sodium lauryl sulphate (SLS)

Stacking gel buffer, (made up at 4 X the required  
final concentration) pH 6.8:

0.5 M 'Trizma' base

0.4 % (W/V) SLS

Electrode buffer, pH 8.3:

25 mM 'Trizma' base

192 mM glycine (Sigma)

0.1 % (W/V) SLS

N,N,N',N' - Tetramethylethylenediamine (TEMED) (Sigma)

10 % (W/V) Ammonium persulphate (AMPS) (BDH)

0.001 % (W/V) bromophenol blue



Coomassie blue staining solution

0.1 % (W/V) Brilliant Blue R (Sigma)

50 % methanol

7 % acetic acid

Destaining solution - 8 % acetic acid, 25 % ethanol

SLS polyacrylamide gel electrophoresis was carried out according to the procedure of Laemmli (1970). 40 ml of 12 per cent running gel was made up from 16 ml of stock (30 per cent) acrylamide, 10 ml of running gel buffer (4 X) and 14 ml autoclaved distilled water, degassed, and 20  $\mu$ l TEMED and 60  $\mu$ l AMPS added. The gel was immediately poured to a height of 17 cm between two acetone-cleaned glass plates. The plates were spaced so as to give a gel thickness of 1 mm. The gel was overlain with water-saturated iso-butyl alcohol (BDH) and left to polymerise for 1 h. The isobutyl alcohol was removed with filter paper and replaced with running gel buffer. This was removed and replaced three times at 15 min intervals, and finally replaced again. The gel was left to fully polymerise overnight.

12 ml of stacking gel was made up from 2 ml stock (30 per cent) acrylamide, 3 ml stacking gel buffer (4 X) and 7 ml autoclaved distilled water, degassed, and 12  $\mu$ l TEMED and 36  $\mu$ l AMPS added. The running gel buffer was removed from the surface of the running gel, and the stacking gel poured on top. The 'comb', consisting of plastic teeth designed to form wells in the stacking gel,





was pushed down into the stacking gel. 25 min later it was gently removed and the wells of the stacking gel washed with electrode buffer.

The gel was placed in the gel tank, both compartments of which contained electrode buffer. The samples, prepared as described in Section 2.8.1, were loaded into the wells of the stacking gel using a Hamilton micro-syringe. The proteins were then electrophoresed at 20 mA and a maximum of 350 V for approx. 5 h (until the dye front was 1 cm from the gel base). The electrode buffer in the lower tank acted as coolant whilst the electrophoresis took place.

Gels of extracts from petals and leaves greater than 1 cm long were stained in Coomassie blue for 30 min, destained for 1.5 h, and dried down on to Whatman 3MM filter paper with a heat lamp and suction plate.

Gels of extracts from primordia were silver stained according to the method of Merril, Goldman, Sedman and Ebert (1981), and dried down as described above.



CHAPTERS 3-6

RESULTS



## CHAPTER 3: DEVELOPMENT OF THE EXPERIMENTAL SYSTEM

### 3.1 Obtaining developmental uniformity

A series of preliminary studies was carried out to find a method of reducing variability in the total number of leaves plus primordia that had been initiated by plants by the time experimental treatments began. Dissection of seeds imbibed in 70 per cent ethanol showed that all contained seedlings with two cotyledons and the first two leaf primordia, regardless of seed weight before imbibition. To test whether seed weight was correlated with the rate of seedling growth, and hence whether variation resulting from this could be eliminated by selection for a narrow range of seed weights, the weights of 32 seeds were recorded before imbibition in water for 24 h. Then the seeds were sown at a depth of 1 cm in Levington potting compound, and placed in LD for 14 d. The plants were harvested and the total number of leaves plus primordia on each plant was counted after dissection under a dissecting microscope. The poor correlation between seed weight and the total number of leaves plus primordia initiated by this time (Fig. 5) suggested that the variation in plant development was not due to the range of seed sizes used. However, measurements of plant height (Fig. 6) and the length of the first leaf (Fig. 7A) showed that both provide a good indication of the total number of leaves plus primordia initiated, and could therefore be used as a basis on which to select a uniform population of seedlings at the beginning of experimental treatments. Since the length of the first leaf showed the closest



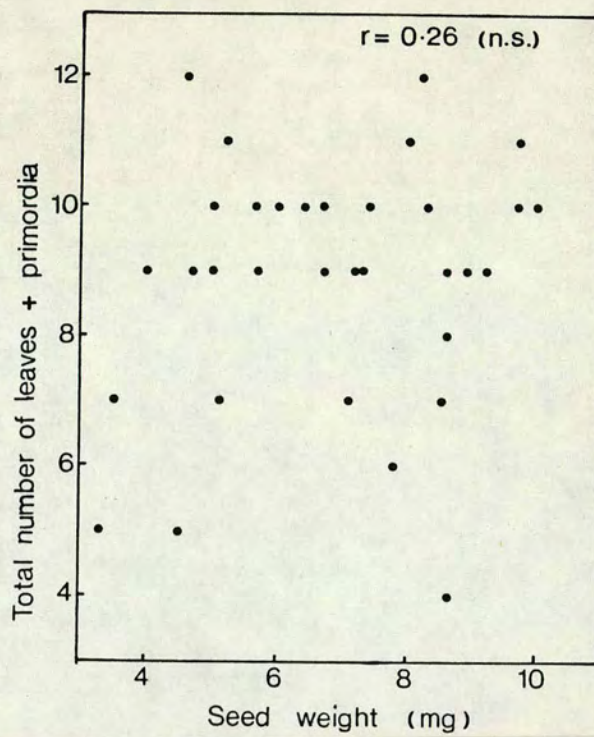


Figure 5. Total number of leaves plus primordia 14 days after sowing, as a function of initial seed weight.  $r$  is the correlation coefficient. n.s.: not significant,

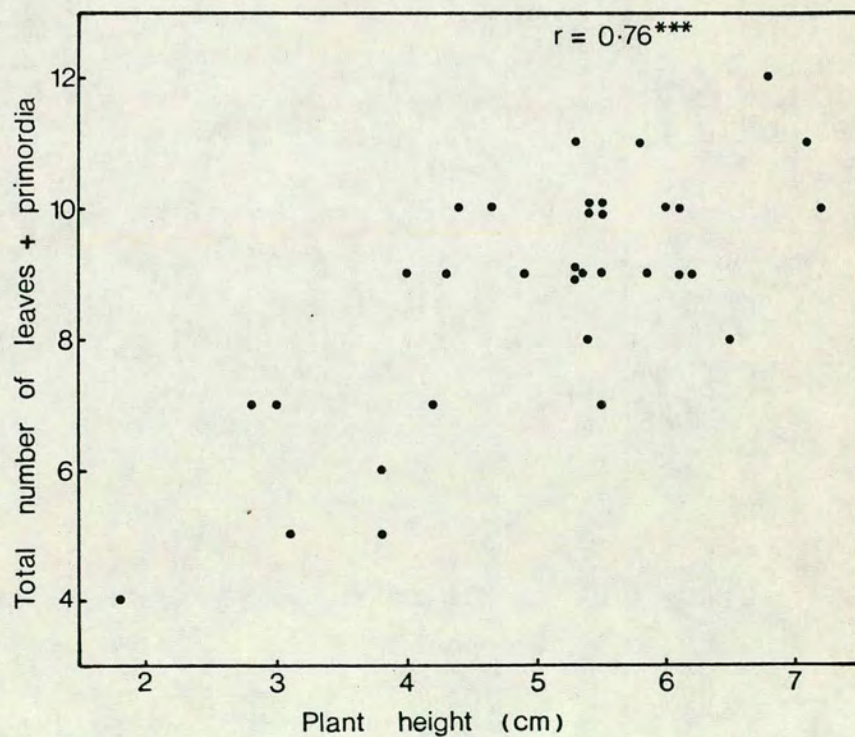


Figure 6. The relationship between total number of leaves plus primordia and plant height 14 days after sowing.

\*\*\*: significant at  $P=0.001$ ,



correlation with the total number of leaves plus primordia initiated (see Figs. 6 and 7A), selection for plants in which the first leaf was between 4 and 8 mm long 7 d after sowing was incorporated into the experimental procedure. An example of the degree of uniformity obtained is given in Figure 7B.

### 3.2 The response to SD and LD

In LD most plants remained vegetative (see, for example, Fig. 8A). In SD the variety of Impatiens used typically formed zygomorphic lateral flowers and also a large actinomorphic terminal flower. It was easiest to study flowering and reversion at this terminal meristem because it was more easily accessible (for dissection and sectioning) than the lateral flowers, and because all its component parts were initiated after the transfer to SD. The terminal flower was usually composed of between three and five bracts, followed by about 20 petals, 20 stamens and five carpels (see, for example, Fig. 8B). In some plants carpels, or stamens and carpels, were not formed. In these cases petals continued to be initiated instead.

Transfer of plants from SD back to LD, before initiation of the terminal flower was complete, caused this flowering terminal meristem to revert to leaf production in most cases. The types of reversion obtained (Table 6) ranged from R1, in which the only sign of an effect of SD was a zone of leaves with no, or modified, axillary structures (the reversion zone), through R4, in



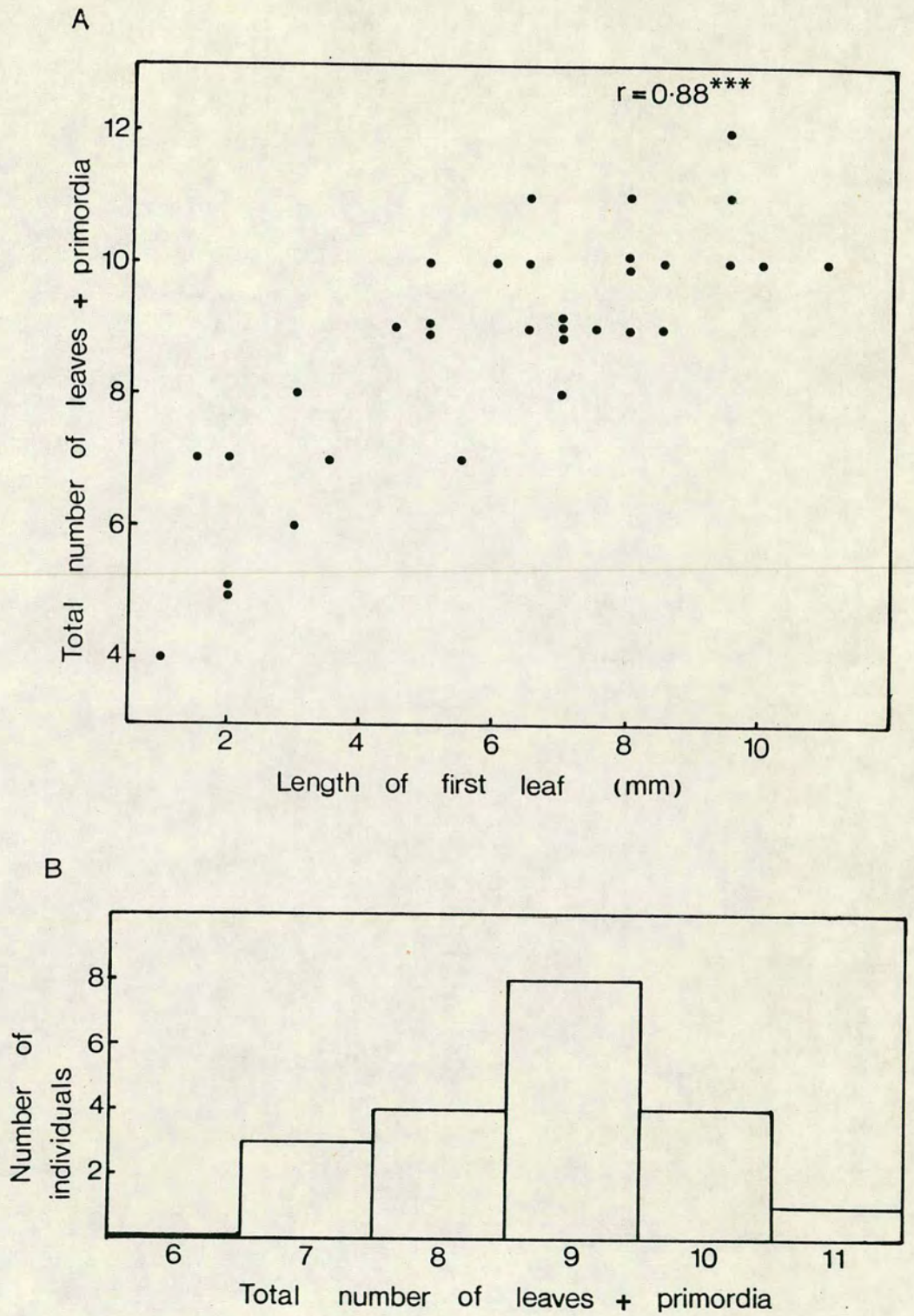


Figure 7.

7(A) The relationship between length of the first leaf, measured from the leaf tip to the abaxial insertion point of the leaf on the stem, and total number of leaves plus primordia 14 days after sowing

7(B) Number of individuals with 6, 7, 8, 9, 10 and 11 leaves plus primordia.



A



B



Figure 8. Illustrations of *I. balsamina*  
(A) growing vegetatively in LD, and  
(B) with a normal terminal flower formed in SD





Figure 8(C) Illustration of intermediate parts from a reverted (R4) plant given 5SD+LD.



TABLE 6. Characteristics of reversion types obtained as a result of transfer of plants back to LD after 3-10 SD

Reversion Type	Characteristics of plants on dissection 6 weeks after return to LD
R0	Plants show no signs of flowering and subsequent reversion
R1	The only sign of flowering is a zone of leaves with no, or modified, axillary structures (the reversion zone)
R2	Zone of leaves with virescent lateral flowers of the terminal inflorescence, above which is a zone of leaves with no axillary structures
R3	As above, but with reduction of internodes between the leaves with no axillary structures
R4	As above, but the leaves of the reversion zone not separated by internodes, and some have modified venation and/or areas with pigment typical of petals. Subsequent leaves in a whorled or pseudo-whorled arrangement, separated by internodes
R5	As above, but a zone of petals occurs before return to leaf production
R6	As above, but with a zone of stamens after the petals, followed by a return to leaves, or occasionally by primordia, either leaves or petals, whose exact nature cannot be determined by inspection
R7	As above, but the zone of stamens is followed by a return to petals



which a zone of intermediate parts (leaves with some pigmented areas - see Fig. 9B, and Fig. 8C) was followed by a return to leaf production in modified arrangement, to the R6-R7 types. In both R6 and R7 petals and stamens were formed and were followed by leaves in R6 or petals in R7.

How far terminal flower formation progressed before reversion depended on the number of SD the plant had received (Table 7). With an increased number of SD there was a decreased proportion of reverting plants of the R4 type, and an increased proportion of R5 to R7 types. However, in all the treatments a number of plants either flowered normally or showed reversion types other than the modal type. In the next section experiments aimed at improving the uniformity of the reversion response will be described.

### 3.3 Improving the uniformity of the reversion response

#### 3.3.1 The effect of altering the growth conditions

A series of experiments was carried out in which the number of leaves and primordia initiated at the beginning of the SD treatment, growth temperature, the type of long day, the number of SD, and the amount of nutrients available to the plants, were varied. The aim of these experiments was to see whether slight alterations in growth conditions resulted in a more uniform reversion response. The results are presented in Table 8. Although in all cases a limited number of SD brought about reversion in most of the plants, the reversion response was no more



TABLE 7. Effect of different numbers of SD on the proportion of plants showing each reversion type. R4, 5 ... = Reversion type 4, 5 ... (no plants showed R0-3 reversion types).  
Combined data from 2 experiments

Number of SD before return to LD	Number of plants					
	R4	R5	R6	R7	Flowering and not reverting	Total
5	13	2	1	1	6	23
7	4	7	3	2	11	27
8	2	11	1	1	11	26
10	2	3	1	3	9	18



Notes to Table 8.

- \* These plants appeared to have reverted to leaf initiation and then produced primordia with petal shape.
- # These plants reverted to leaf or leafy-petal initiation, and then produced petal and stamen primordia.
- \*\* LD+ consisted of LD with 2 h fluorescent + tungsten (total photosynthetic photon flux density 275-285  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  at the top of the plants on day 0) in the middle of the 16 h tungsten period.
- ## These plants received standard experimental conditions (see Section 2.2) except that they were grown in sand and supplied with Hoagland's solution only once per week.



TABLE 8. Effects of different experimental regimes on the numbers of plants showing each reversion type. R1, 2 .... Reversion type 1, 2 .....

Treatment		Number of Plants											
Temperature of LD before SD ( <sup>o</sup> C)	Number of leaves + primordia at beginning of SD	Number of SD	Type of long day after SD (Temp. <sup>o</sup> C)	R1	R2	R3	R4	R5	R6	R7	Flowering and not reverting	Total	
22+1	11-13	7	LD (22+1)				3	2	1		1	7	
20+1	11-13	7	LD (22+1)				2	3			2	7	
22+1	11-13	7	Continuous light (20+1)				3	2			2	7	
20+1	11-13	7	Continuous light (20+1)				1	3	1		2	7	
22+1	8-10	8	Continuous light (20+1)			1	2*+1	2#				6	
22+1	8-10	5	Continuous light (20+1)		1	3	1*					5	
22+1	8-10	5	LD+** (22+1)			2	3				1	6	
#22+1	8-10	5	LD (22+1)		1		2	2		2		7	



uniform than in the standard regime (compare with Table 7). The characteristics of the reversions obtained were similar to those previously described (see Section 3.2), except that SD followed by continuous light resulted in reversion followed by a return to petal or stamen initiation in some plants (Table 8), a phenomenon never observed in SD followed by LD.

Since altering the growth conditions did not improve the uniformity of the reversion response, the following alternative method was adopted.

3.3.2 The relationship between flower colour and reversion response; its use in obtaining uniform reversions

5SD + LD caused the most uniform reversion response (Table 7). In the R4 type that resulted from this treatment in most cases, the lack of internodes in the reversion zone and the presence of intermediate parts was evidence that flowering had begun, but leaf initiation and internodal development resumed more quickly than in the R5-R7 reversion types. For these reasons it was decided to concentrate attention on this treatment, and to increase uniformity so that all, or nearly all, plants treated in this way showed the R4 reversion type.

The seed of Impatiens used for the work reported in this thesis gave rise to a population of plants with purple, pink, red, white and magenta flowers. Each individual plant bore flowers of only one colour. Observation of the flower, or reverted flower, colours of plants given 5SD + LD indicated that the red-flowered plants tended to



show the R4 reversion type much more frequently than plants with other flower colours. This is demonstrated in Table 9, in which the reversion response of plants grouped according to flower colour is shown. Clearly, it would be highly desirable to carry out experiments on a population made up of entirely red-flowered plants.

Since seed giving rise to only red-flowered plants could not be obtained, in later experiments analyses were restricted to red-flowered plants as described in Section 2.5. Only the characteristics of red flowers, or reverted flowers, were scored at maturity in these experiments. That flower colour is a stable feature, unaffected by removal of the terminal shoot, is demonstrated by the fact that the proportion of red-flowered plants was the same in control plants forming a terminal flower in SD and in plants which had regenerated from the axillary meristems in SD (Table 10).



TABLE 9. The proportion of plants, grouped into flower colours, showing each reversion type in response to 5SD+LD. R3, 4 ..... = Reversion type 3, 4, ..... (no plants showed R0-2 reversion types). Data for red-flowered plants combined from 10 experiments; data for other flower colours combined from 6 experiments

Flower colour	Number of plants						Total <sup>#</sup>
	R3	R4	R5	R6	R7	Flowering and not reverting	
Red	6*	32	3	0	1	2	44
White	1	10	5	1	1	1	19
Pink	0	1	0	0	0	11	12
Magenta	0	0	1	0	0	5	6
Purple	0	0	0	0	0	12	12

\* In these plants flower colour could be determined because some parts of the axillary reverted flowers showed areas with red (petal) pigmentation.

<sup>#</sup> In these experiments the flower colour of 11 plants (5R3, 1R5, 1R6 and 4 flowering and not reverting) could not be determined because of lack of development of pigmentation in the petals (R5, R6 and flowering and not reverting) or because no petaloid areas occurred (R3). These are not included in the total.



TABLE 10. Numbers of plants showing red or pink/purple/magenta flowers when grown in SD with and without removal of the terminal shoot. The data from plants in treatment 1 were used to calculate the expected frequencies for treatment 2 and  $\chi^2$  calculated according to Parker (1979)

Treatment	Number of red-flowered plants			Number of pink-, purple- and magenta-flowered plants			Total
1. SD (control plants forming a terminal flower)		46			48		94
	Observed	Expected	Difference	Observed	Expected	Difference	
2. SD (terminal shoot removed and plants regenerated from the axillary meristems)	77	79.8	-2.8	86	83.2	+2.8	163

$$\chi^2 = \frac{(2.8 - 0.5)^2}{79.8} + \frac{(2.8 - 0.5)^2}{83.2} = 0.13, \text{ D.F.} = 1. \quad p > 0.5.$$



## CHAPTER 4: FLOWERING IN SD AND REVERSION IN 5SD + LD

The experiments described in this chapter were carried out using all plants which showed a terminal flowering response to SD. In these experiments, although most of the reverting plants in <sup>the</sup> 5SD + LD treatment will be R4 reversion types (see Table 7), the data will inevitably include other reversion types and non-reverting plants. However, those statements concerning the morphology of reversion refer to the R4 type exclusively.

### 4.1 Morphological characteristics of flowering and formation of the R4 reversion type

#### 4.1.1 The terminal meristem

Examination of over 200 plants showed that in continuous SD the terminal flower was formed as follows. At the time of transfer to SD about 8-9 leaves plus primordia were present. Part 9 or 10 (numbering from the first leaf upwards) was usually the first to show differences from normal leaves, since this and all subsequent parts lacked axillary structures, and one or both of the basal pair of second order veins were not fused to the main vein in the lamina, but retained an independent identity into the petiole (Fig. 9A). Parts 10-14 usually showed increasing degrees of such modified venation, the second pair of second order veins being affected in a similar way to the basal pair. These parts also showed progressive reduction in size compared with normal leaves, often had small areas of coloured pigment characteristic of petals, generally lacked a petiole, and also often had a







spur. Those parts which were less than 50 mm long when mature, and which lacked a petiole and/or had a spur, are referred to as bracts. The first petal was usually part 15, and is defined as the first part lacking a spur which had more than 50 per cent of its area pigmented. Successive petals became more pigmented until the maximum amount of pigmentation (about 85 per cent of the petal) was attained by about part 17.

The modified leaves and bracts were initiated during the first 5SD. Petals were initiated from the 5th SD onwards, until the formation of the first stamen (about part 34-35) on about the 12th SD. Carpel formation began on about the 19th SD (about part 56-57).

These features of terminal flowers formed in SD are summarized in Table 11.

In plants showing the R4 reversion type in response to 5SD + LD, organs without an axillary structure and with modified venation were initiated, as in plants flowering in SD (Fig. 9B; Table 11). Subsequently, intermediate parts (parts with some areas of petal pigment) developed, instead of normal petals (Fig. 9B; Table 11). As is the case with petals in the normal flower, these parts were not separated by internodes at maturity. At about the same time that normal leaves began to be initiated again, internodal development resumed (Table 11); these leaves were initiated in whorls or pseudo-whorls, usually of about 5-6 parts. After about 5-6 such whorls or pseudo-whorls had been initiated, the apex returned to spiral phyllotaxis. At the same time axillary meristems



TABLE 11. Summary of characteristic features of terminal flowers (SD) and reversions of the R4 type (5SD+LD). Combined data from 10 experiments

Treatment	Mean first part (numbering from base upwards) with the following feature(s) (+ 1 s.e.)								
	No axillary structure	Modified venation	Bract	Petal pigment	Petal	Stamen	Carpel	Without petal pigment (leaf)	Above which internode is $\geq 1$ mm long
SD	9.0+0.1	9.7+0.2	11.5 +0.1 _	13.3 +0.2 _	15.1 +0.2 _	34.4 +0.8 _	56.1 +1.2 _	-	-
Number of plants	77	66	74	49	76	78	68	-	-
5SD+LD (R4)	9.1+0.2	10.4+0.2	-	15.5 +0.3 _	-	-	-	25.0+0.6	24.5+0.7
Number of plants	39	39	-	39	-	-	-	38	39



were again initiated in the leaf axils.

#### 4.1.2 The axillary meristems

The axillary meristems subtended by leaves 1 to 8 or 9 also showed a flowering response when plants were transferred to SD on day 0. Meristems in the axils of leaves 1 and 2 grew out as lateral branches with terminal flowers. The lower leaves of these branches bore zygomorphic axillary flowers. Meristems in the axils of mainstem leaves 3 to 6 usually initiated two or more zygomorphic flowers; dissection of these meristems revealed that the flowers were initiated in the axils of the (undeveloped) leaves of a suppressed lateral branch. Mainstem leaves 7 to 9 usually bore only a single flower in their axils, which did not originate from the axil of a leaf of a suppressed lateral branch. The pedicel of the flower in the axil of mainstem leaves 8 and 9 was usually fused to the mainstem. Under the experimental conditions used here, the terminal inflorescence can therefore be considered to begin at about part 7, and the terminal flower at about part 9-10 (the first leaf without an axillary structure).

When reversion occurred at the terminal meristem it typically occurred at the lateral meristems as well. However, the form of the reversions found at these meristems varied, according to the position of the meristem on the plant. This variation reflected the difference in developmental origin of the flowers that these meristems normally produced in SD. In a plant showing an R4 terminal flower



reversion, the lateral flowers of the terminal inflorescence (usually those in the axils of mainstem leaves 7 to 9) also typically showed the R4 reversion type. In the axils of mainstem leaves 5 to 6, on the other hand, vegetative branches grew out; these differed from normal vegetative branches in that flowers (frequently reverted) were present in the axils of leaves 1 and 2. Completely vegetative branches grew out from the axils of mainstem leaves 1 to 4. In plants showing other reversion types at the terminal meristem, a similar variation was found at the lateral meristems.

#### 4.2 Changes in apical growth and phyllotaxis

##### 4.2.1 Rates of primordium initiation

The rate of primordium initiation during vegetative growth in LD was constant (Fig. 10) and was 0.9 primordia per day (Table 12). In continuous SD the rate of primordium initiation increased during the first 8SD, and between days 8 and 13, when petals and the first stamens were being initiated, increased further to 3.8 primordia per day. During the initiation of stamens (days 13-19) the rate declined to 1.3 primordia per day and then increased to about 3.4 primordia per day before carpel initiation began on day 19 (Fig. 10; Table 12). The most rapid rate of primordium initiation during flowering therefore accompanied the initiation of petals and the earliest and latest stamens.

In plants given 5SD + LD the rate of primordium initiation had increased from the vegetative value



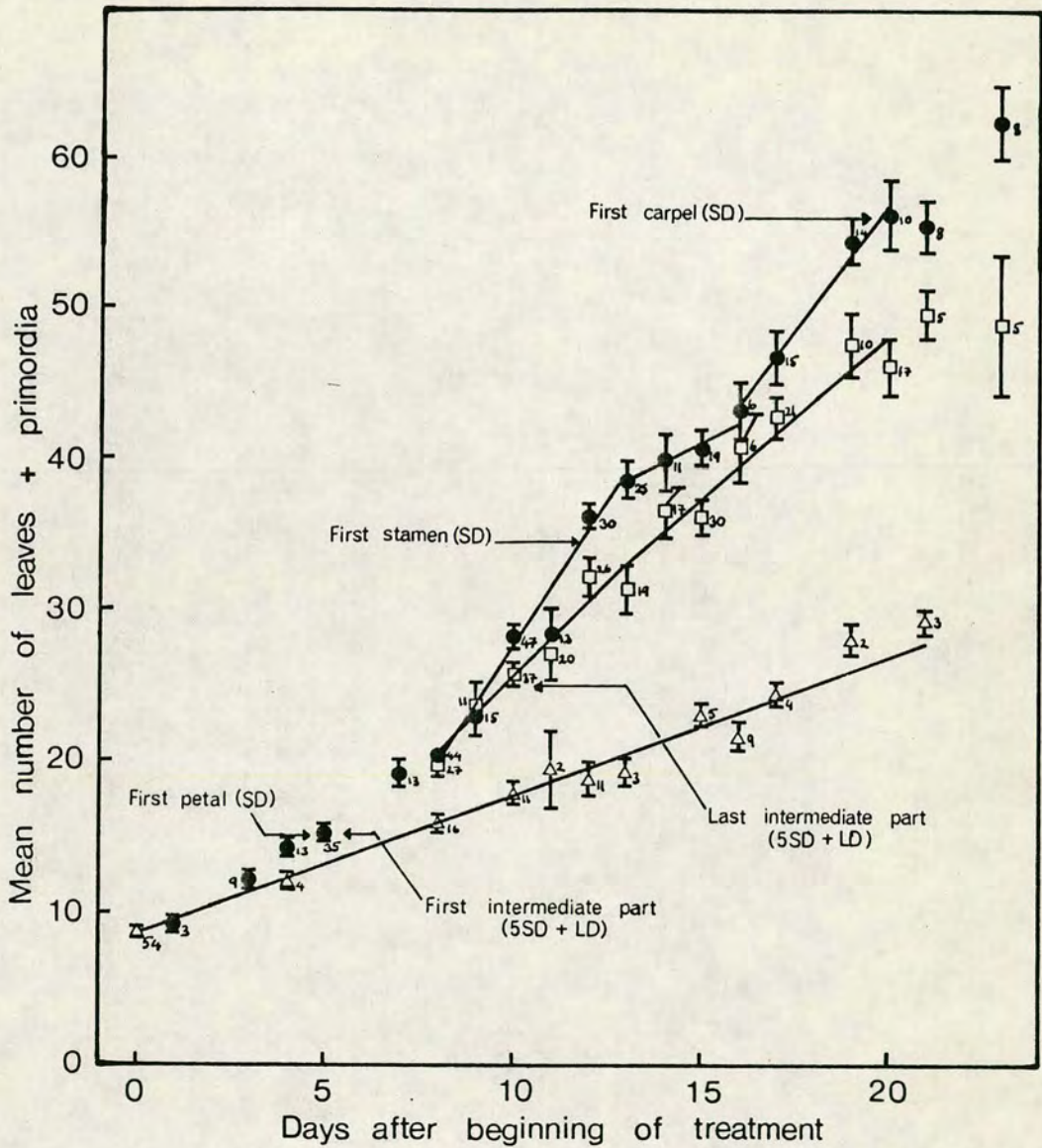


Figure 10. Changes of primordium number in vegetative (LD) ( $\Delta$ ), flowering (SD) ( $\bullet$ ), and reverting (5SD+LD) ( $\square$ ) plants. Combined data from 10 experiments. Mean values  $\pm$  1 s.e. are presented. The number beside each point indicates the number of replicates in each sample. Full lines are those given by regression equations. Times at which changes in initiation rate occur were determined by inspection for the SD treatment. To allow comparison of the rates of initiation from day 8 to day 13 in SD and 5SD+LD, regression lines were fitted from day 8 to day 13, and from day 13 to day 20, in the 5SD+LD treatment. Values of the regression coefficients are given in Table 12.



TABLE 12. Rates of primordium initiation per day during different phases of development of plants growing in LD (vegetative), SD (flowering), and 5SD+LD (reverting). Rates of initiation were estimated as the regression coefficients<sup>a</sup> from regression lines fitted to the data of Figure 10

Treatment	Type of apex	Days after beginning of treatment	Rate of initiation of primordia day <sup>-1</sup> (+ 1 s.e.)
LD	Vegetative	0-21	0.9 $\pm$ 0.03
SD	Flowering	8-13	3.8 $\pm$ 0.3
		13-16	1.3 $\pm$ 0.2
		16-20	3.4 $\pm$ 0.3
5SD+LD	Reverting	8-13	2.5 $\pm$ 0.3
		13-20	2.2 $\pm$ 0.3

<sup>a</sup> Regression analyses were performed throughout using the method given in Sokal and Rohlf (1969)



by day 8, as in flowering plants. After day 8 it did not increase to the same degree as in SD (Table 12), since from day 10 onwards the mean number of leaves plus primordia from plants in 5SD + LD was consistently lower than in plants in SD (Fig. 10). The rate of primordium initiation in 5SD + LD was 2.5 primordia per day between days 8 and 13, and 2.2 primordia per day between days 13 and 20 (Table 12). Therefore, when the reverting plants began to make leaves again (about day 10) the rate of primordium initiation was not quite as rapid as during petal initiation in SD, but was more than twice as rapid as during leaf initiation in vegetative plants in LD. Clearly, there was not a particular rate of initiation associated with leaf production, and reversion did not result in an immediate return to the original vegetative (LD) rate.

However, between days 42 and 74, reverted (R4) plants initiated primordia at about the same rate as vegetative plants (Fig. 11; Table 13). This rate was slightly slower than in vegetative plants at earlier stages of development (compare Tables 12 and 13); the implication is that reverted plants do eventually resume primordium initiation at the same rate as vegetative (LD) plants of the same chronological age. Furthermore, since reverted plants had initiated about 50 leaves plus primordia on day 21 (Fig. 10), and the predicted mean value on day 42 is about 61 leaves plus primordia (Fig. 11), it seems probable that the rate of primordium initiation slowed to the vegetative rate, or perhaps even to a lower rate



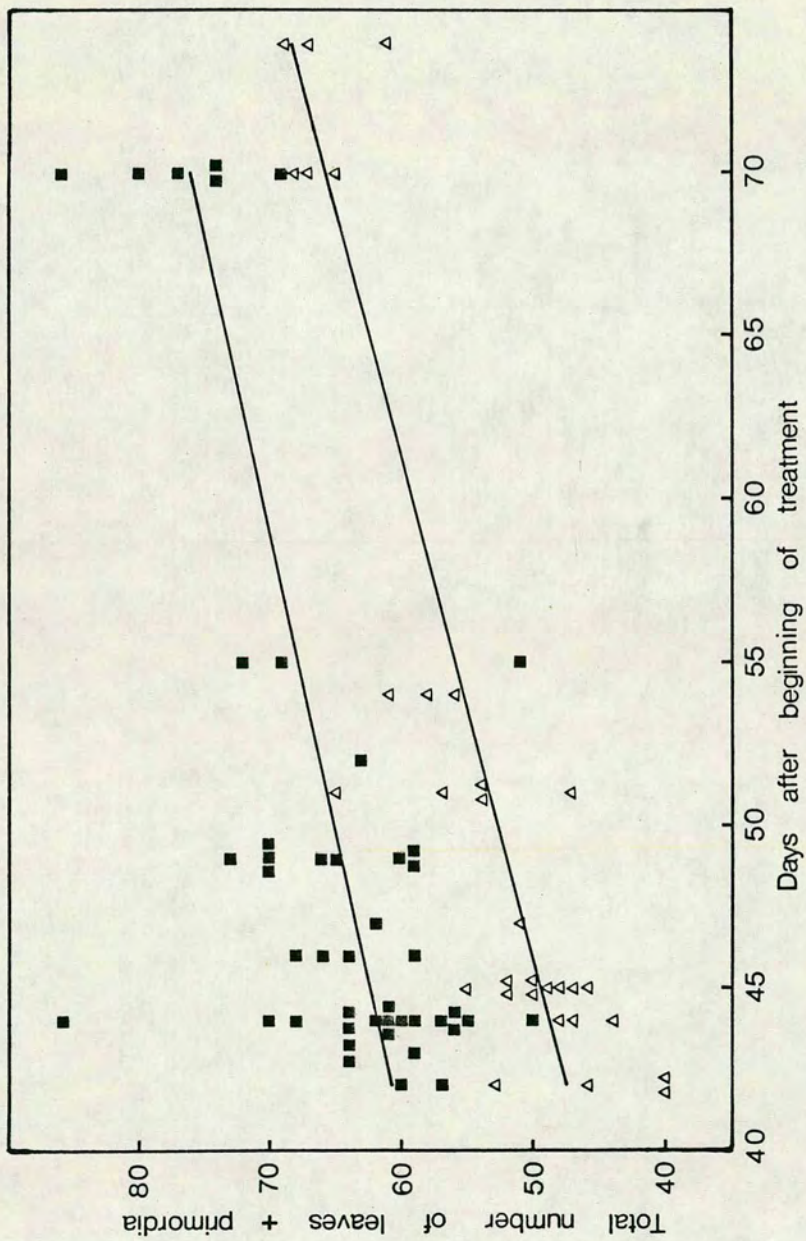


Figure 11. Change of primordium number in vegetative (LD) ( $\Delta$ ), and reverted (R4) (5SD+LD) ( $\blacksquare$ ) plants. Data in each treatment derived from dissection of plants at maturity in successive experiments. Full lines are those given by regression equations. Values of the regression coefficients are given in Table 13



TABLE 13. Rates of primordium initiation between days 42 and 74 after the beginning of experimental treatments in vegetative (LD) and reverted (5SD+LD) plants

Treatment	Type of apex	Rate of initiation of primordia day <sup>-1</sup> ( $\pm$ 1 s.e.)
LD	Vegetative	0.66 $\pm$ 0.07
5SD+LD	Reverted	0.55 $\pm$ 0.06



than this, very soon after day 21.

#### 4.2.2 Divergence angles

Vegetative plants always had spiral phyllotaxis, with a mean divergence angle of  $137.9^{\circ}$  (Table 14; Fig. 12A). During the transition to petal initiation in SD (parts 10-20), and intermediate part initiation in 5SD+LD (parts 10-20), the mean divergence angle was not significantly different from that in vegetative plants (Table 14). At about part 21, whorls or pseudo-whorls, each of about six parts, were formed in both the SD and the 5SD + LD treatments (Fig. 12B and C), even though petals were formed in SD, but intermediate parts in 5SD + LD. A pseudo-whorled or whorled arrangement persisted during subsequent flowering in SD (Fig. 13A) and also on reversion to leaf initiation in 5SD + LD (Fig. 13B) - there was no immediate return to spiral arrangement found in LD (vegetative) plants. However, in plants sampled 6-7 weeks after the beginning of experimental treatments, the reverted apex had typically just switched from a whorled to a spiral arrangement (see, for example, Fig. 14A). This change occurred on average at part  $59.0$  (1 s.e. =  $4.0$ ,  $n=6$ ), initiated on about day 40 (see Fig. 11). By day 70 the vegetative (LD) and reverted (5SD + LD) apices were indistinguishable, both showing spiral phyllotaxis and



TABLE 14. Divergence angles between parts of differing morphology initiated in LD, SD and 5SD+LD. Apices that had initiated between 14 and 38 leaves + primordia were used to measure divergence angles for parts 10-15 and 15-20 in SD and 5SD+LD. Apices that had initiated between 14 and 26 leaves + primordia were used to measure divergence angles for parts 10 onwards in LD. In 5SD+LD (mature) and LD (mature) plants divergence angles were measured from the 11 youngest leaves. In all cases divergence angle was measured according to the method of Erickson & Maksymowych (1977)

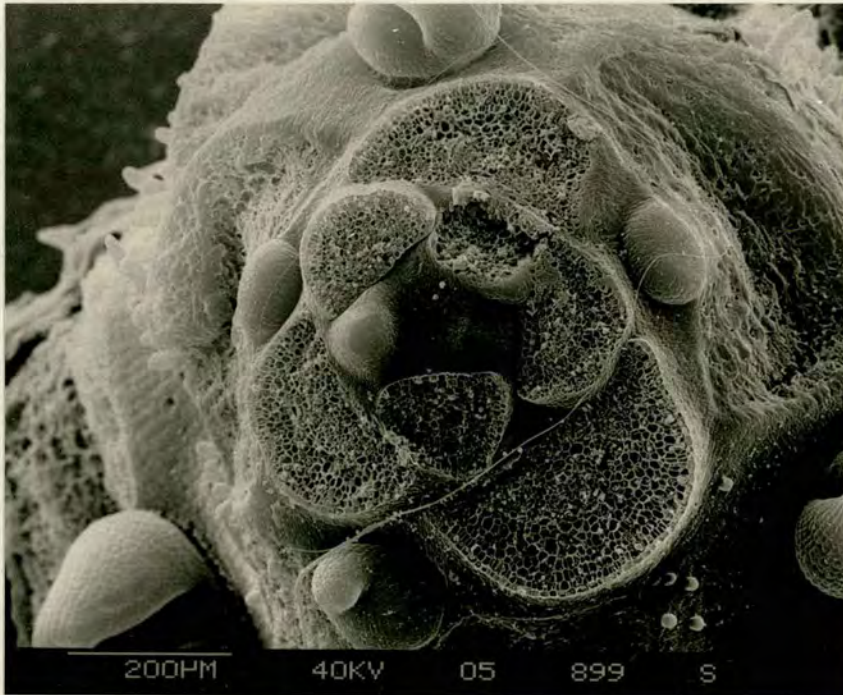
Treatment (parts measured)	Number of apices	Number of measurements	Mean divergence angle(°) $\pm$ 1s.e.
LD (leaves)	10	76	137.9 $\pm$ 0.6
SD (bracts) (parts 10-15)	12	56	139.6 $\pm$ 1.0
SD (petals) (parts 15-20)	9	37	139.4 $\pm$ 1.4
5SD+LD (reverted bracts) (parts 10-15)	11	51	137.1 $\pm$ 1.5
5SD+LD (intermediate parts) (parts 15-20)	13	61	136.1 $\pm$ 1.0
5SD+LD (mature) (leaves)	2	18	138.4 $\pm$ 1.1
LD (mature) (leaves)	3	27	138.2 $\pm$ 0.7

Analysis of variance on all 7 groups - n.s.

Analyses of variance were performed throughout using the method given in Sokal and Rohlf (1969)



A



B



Figure 12. S E M photographs of the shoot apex of plants sampled after (A) 12LD (total number of leaves + primordia = 19), (B) 10SD (Total number of leaves + primordia = 37).



C



Figure 12(C). S E M photograph of the shoot apex of a plant sampled after 5SD+7LD (total number of leaves + primordia = 32).





Figure 13. Transverse sections grazing the apical surface of plants sampled after  
(A) 19SD (total number of leaves + primordia = 58), and (B) 5SD+12LD (total number of  
leaves + primordia = 38)



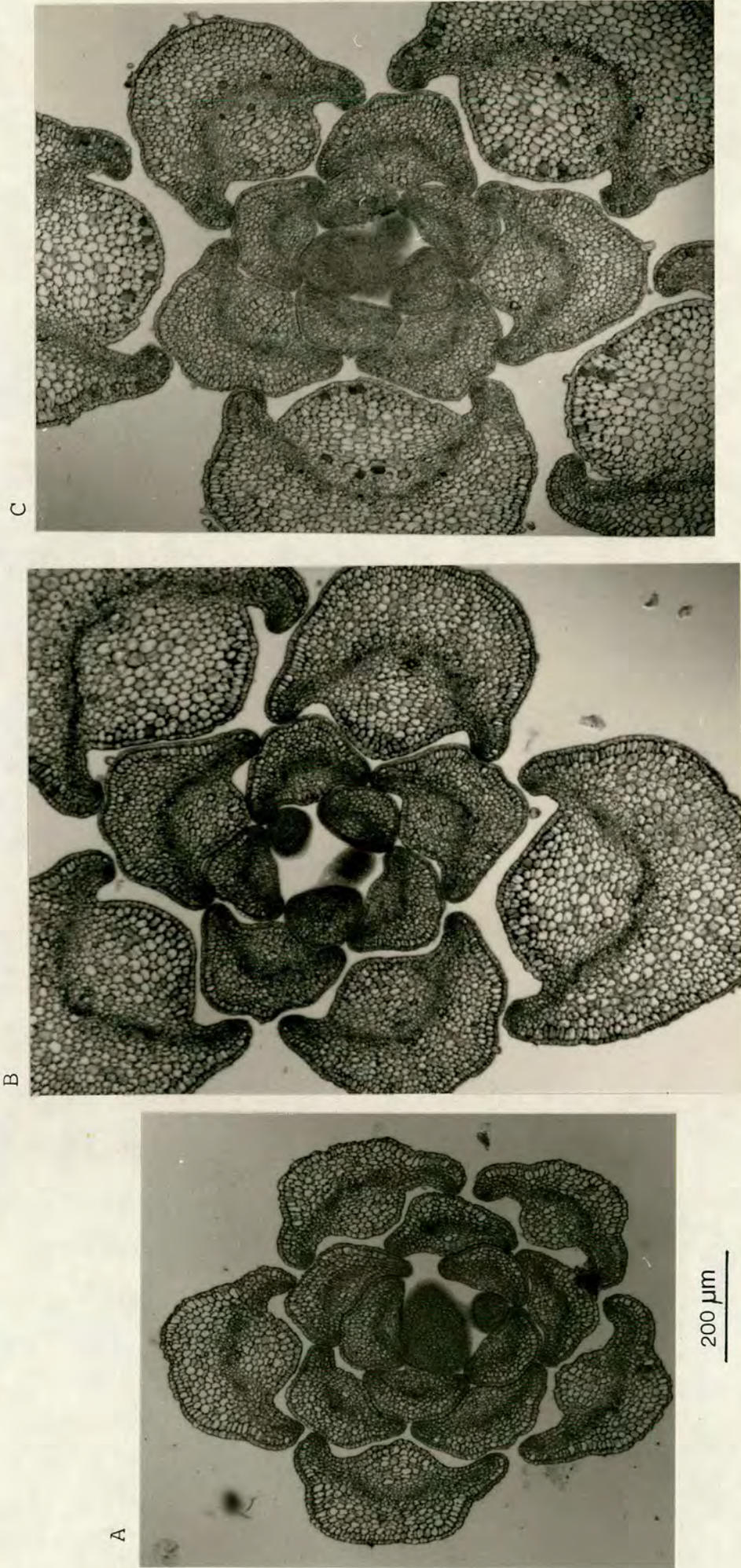


Figure 14. Transverse sections grazing the apical surface of plants sampled after (A) 5SD+39LD (total number of leaves + primordia = 56), (B) 74LD (total number of leaves + primordia = 67), (C) 5SD+65LD (total number of leaves + primordia = 77)



having divergence angles of about  $138^{\circ}$  (Fig. 14B, C; Table 13).

#### 4.2.3. Area of the apical surface and areas of primordia at initiation

##### A. Early flower development and reversion (days 0-12)

In vegetative, flowering and reverting plants the youngest primordium was initiated at about the same distance from the apical centre (Table 15); hence the bare area of the apical surface,  $\pi \times (\text{mean radial distance of the youngest primordium from the apical centre})^2$ , remained constant at about  $9 \times 10^3 \mu\text{m}^2$ . The total area of the apical surface,  $\pi \times (\text{mean radial distance of the first primordium below the apical surface})^2$ , also remained constant at about  $41 \times 10^3 \mu\text{m}^2$  in the three treatments. This implies that the area of the annulus bearing the primordia on the apical surface is also constant, and this is what was found (Table 15). However, the number of primordia occupying this area increased to about the same extent in both the flowering and the reverting apices, so that there were 2-3 times as many primordia on the apical surface as on that of a vegetative plant. The implication is that the primordia must be smaller in the flowering and in the reverting apices.

Using the plastochron ratio ( $\underline{r}$ ), the area of a primordium at initiation, relative to the area of the apex, can be calculated as  $2\log_e \underline{r}$  (the inverse of the area ratio according to Richards, 1951). Since the apical surface on which the primordia were initiated remained flat



TABLE 15. Area of the apical surface and areas of primordia on initiation during different phases of development of vegetative (LD), flowering (SD), and reverting (5SD+LD) plants sampled between days 0 and 12 after the beginning of experimental treatments (all data obtained from SEM photographs except where indicated)

Treatment	Type of apex	Stage of development (total no. of leaves + primordia)	Number of plants per sample	Mean distance from the apical centre of the youngest primordium ( $\mu\text{m}$ ) <sup>a</sup>	Area of annulus bearing primordia on apical surface ( $10^3 \times \mu\text{m}^2$ ) <sup>b</sup> (+ 1 s.e.)	Number of primordia on the apical surface	Area of primordium at initiation relative to area of apical surface ( $2 \log r$ ) <sup>d</sup>	Absolute area of primordium at initiation (intercept at age 0 of regression of log (basal area of primordia) against primordium age in plastochrons) ( $10^3 \times \mu\text{m}^2$ )
LD	Vegetative	Leaf	10	59.5	28.3+2.7	3	0.41	4.7
								3.6
SD	Flowering	Mid-petal (21-29)	3	48.9	29.1+9.9	7	0.23	1.8
								1.3
		Late petal (30-38)	7	44.6	37.8+5.4	8	0.26	1.7
								1.6
5SD+LD	Reverting	Intermediate part/leaf (21-29)	7	60.5	27.5+3.9	7	0.20	2.4
								1.4
		Leaf (30-34)	5	49.1	43.3+6.9	10	0.20	1.6
								1.6

<sup>a</sup> Geometric means presented; analysis of variance on log transformed data n.s.  
<sup>b</sup> Analysis of variance on all 5 treatment stages: n.s.  
<sup>c</sup> Data obtained from serial transverse sections; see Table 18 for number of plants per sample.  
<sup>d</sup> Analysis of variance on all 5 treatment stages: LD plants significantly different from remaining plants at  $p=0.005$ .



between days 0 and 10 in LD, SD and 5SD + LD (Fig. 15), and observation of longitudinal sections cut for other purposes (see Section 6.2, p. 194), serial transverse sections, and freshly dissected apices, all indicated that the apex of I. balsamina was always flat, the apical angle is  $180^{\circ}$ . This means that  $2\log_e r$  requires no correction as is usually necessary with domed apices (Richards, 1951). In both the flowering and the reverting plants the area of a primordium at initiation relative to the area of the apical surface, calculated in this way, decreased significantly from that in the vegetative apex (Table 15). The absolute area of a primordium on initiation also decreased on flowering and remained lower on reversion. Confirmation of this trend was obtained when the area of the primordium on initiation was estimated from regression lines fitted to graphs of  $\log_{10}$  (basal area of the primordium) against primordium age in plastochrons (Fig. 16; Table 15).

On flowering there was therefore no change in the area of the apical surface, but there was a reduction in the absolute area of the primordia initiated. This reduction in area was maintained when the plant reverted to leaf initiation, indicating that the area of the primordium at initiation does not determine its developmental fate.

#### B. Later development of the flowering and reverted apices (days 11 - 21)

The distance of the youngest primordium from the apical centre remained unchanged during later stages of



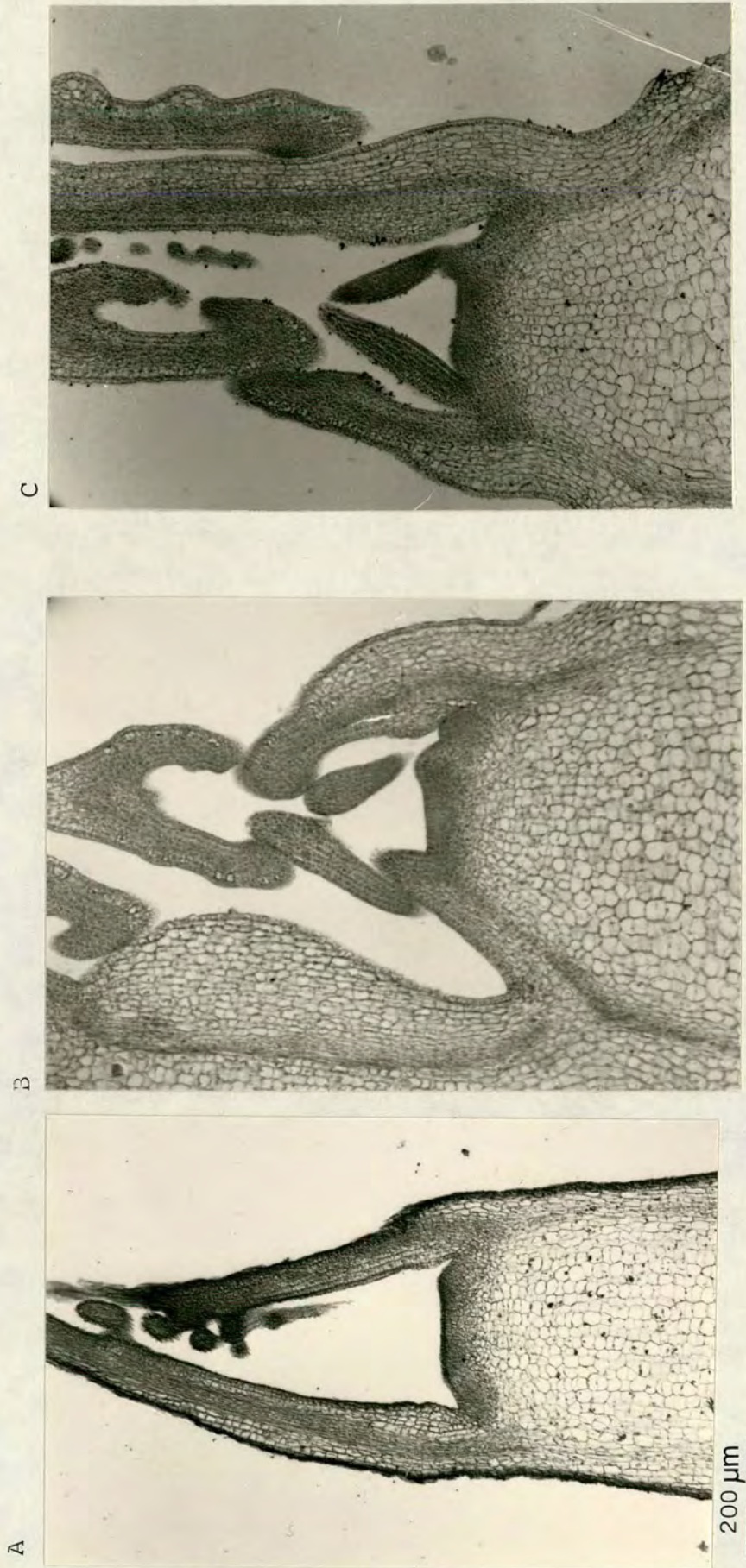


Figure 15. Longitudinal sections of the shoot apex of plants sampled (A) on day 0 (7 days after sowing), (B) after 3LD, (C) after 10 LD, illustrating the flat apical surface of vegetative I. balsamina.





Figure 15. Longitudinal sections of the shoot apex of plants sampled (D) after 3SD, (E) after 10 SD, and (F) after 5SD+5LD, illustrating the flat apical surface of flowering and reverting I. balsamina.



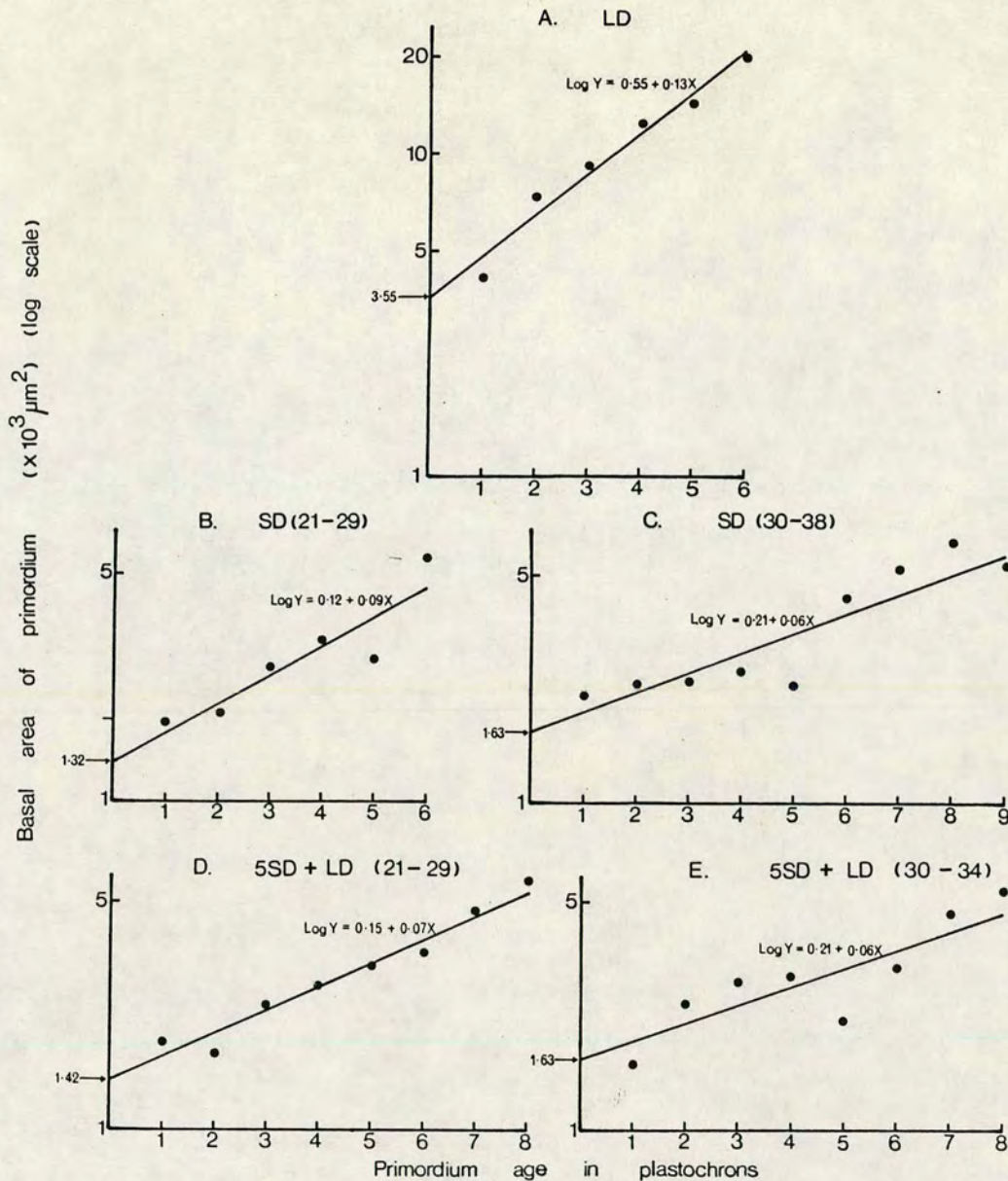


Figure 16.  $\text{Log}_{10}$  (basal area of primordium) as a function of primordium age in plastochrons, for: (A) vegetative (LD), (B) flowering (SD) (21-29), (C) flowering (SD) (30-38), (D) reverting (5SD+LD) (21-29), and (E) reverting (5SD+LD) (30-34), apices. (Numbers in brackets are the total number of leaves + primordia at each developmental stage.) Each point is the mean of at least 3 values; regression equations are shown. Arrows indicate the estimated area of the primordia at initiation in each treatment stage (see also Table 15).



development of plants in the three treatments (Table 16). However, the first primordium below the apical surface was initiated nearer the apical centre in the flowering and reverted apices than in the vegetative apex. This resulted in a decrease in the area of the annulus bearing the primordia on the apical surface between the Late petal stage of flowering and the Leaf (30-34) stage of reverting apices (Table 15), and the subsequent stages in both types of apex (Table 16). However, the number of primordia on the apical surface decreased from eight at the Late petal stage (Table 15) to five at the Stamen/carpel stage (Table 16) of the flowering apex, and from 10 at the Leaf (30-34) stage (Table 15) to six at the Leaf (34-49) stage (Table 16) of the reverted apex. Because both the area of the annulus and the number of primordia occupying this annulus decreased in this way, the area of the primordium at initiation might be expected to be about the same at the end of flowering or after reversion as during earlier stages of flower development or during reversion. This is what was found when the plastochron ratio ( $\bar{r}$ ) was used to estimate the area of the primordium on initiation, relative to the area of the apical surface (Table 16), although the difference between vegetative (LD) and flowering (SD) apices at the Stamen/carpel stage was not as pronounced as it was between these / <sup>two</sup> types of apex at earlier stages of flower development (compare Tables 15 and 16). The absolute area of the primordia initiated by plants in SD and 5SD + LD remained markedly lower than in vegetative (LD) apices (Table 16).



TABLE 16. Area of the apical surface and areas of primordia on initiation in vegetative (LD), flowering (SD), and reverted (5SD+LD) plants sampled between days 11 and 21 after the beginning of experimental treatments (all data obtained from transverse sections of the shoot apex)

Treatment	Type of apex	Stage of development (total no. of leaves + Primordia)	Number of plants per sample	Mean distance from the apical centre of the youngest Primordium ( $\mu\text{m}$ ) <sup>a</sup>	Area of annulus bearing primordia on apical surface ( $10^3 \times \mu\text{m}^2$ ) <sup>b</sup>	Number of primordia on the apical surface	Area of primordium at initiation relative to area of apical surface ( $2\log_e$ ) <sup>c</sup>	Absolute area of primordium at initiation (apical area $\times 2\log_e$ ) ( $10^3 \times \mu\text{m}^2$ )
LD	Vegetative	Leaf	6	68.0	31.9+6.1	3	0.46	6.7
SD	Flowering	Stamen/carpel (44-58)	5	50.2	26.3+2.1	5	0.32	2.5
5SD+LD	Reverted	Leaf (34-49)	6	48.8	22.1+3.5	6	0.23	1.8

<sup>a</sup> Geometric means presented.

<sup>b</sup> Analysis of variance n.s.

<sup>c</sup> Analysis of variance: LD plants significantly different from remaining plants at  $P=0.05$ .



Flowering and reverted apices therefore initiated primordia of similar absolute area at initiation, on apices of similar area, even though in the flower the primordia developed as stamens or carpels, whilst in the reverted flower they developed as leaves. The reverted apex remained very dissimilar to the vegetative (LD) apex, even though in both cases the primordia initiated developed as leaves.

C. The mature reverted (5SD + LD) and vegetative (LD) apices (days 70-74)

The preceding studies indicated that the reverted apex resumed initiation of leaves after about day 10, but that its phyllotaxis and radial growth pattern remained very similar to that found in the flowering (SD) apex. The data presented in Table 17, and the illustrations in Figure 14B and C, show that, about 10 weeks after the beginning of experimental treatments, the vegetative (LD) and reverted (5SD + LD) apices were indistinguishable from one another. Furthermore, since the vegetative (LD) apex was also indistinguishable from vegetative apices sampled between days 0 and 12 (compare Tables 15 and 17), it is clear that the reverted apex eventually returned to the original vegetative pattern of radial growth.

4.2.4 Lengths of stem frusta on initiation

A. Early flower development and reversion (days 0 - 12)

Just as the area of a primordium at initiation (relative to the area of the apex) is  $2 \log_e r$ , where



TABLE 17. Area of the apical surface and areas of primordia on initiation in vegetative (LD) & reverted (R4) (5SD+LD) plants sampled 70-74 days after the beginning of experimental treatments (all data obtained from transverse sections of the shoot apex)

Treatment	Type of apex	Stage of development (total no. of leaves + primordia)	Number of plants per sample	Mean distance from the apical centre of the youngest primordium ( $\mu\text{m}$ ) <sup>a</sup>	Mean distance from the first primordium below the apical surface ( $\mu\text{m}$ ) <sup>a</sup>	Area of annulus bearing primordia on apical surface ( $10^3 \times \mu\text{m}^2$ ) (+ 1 s.e.) <sup>b</sup>	Number of primordia on the apical surface	Area of primordium at initiation, relative to area of apical surface ( $2 \log r$ ) <sup>b</sup>	Absolute area of primordium at initiation (apical area $\times 2 \log r$ ) ( $10^3 \times \mu\text{m}^2$ )
LD	Vegetative	Leaf	3	62.4	114.6	28.9 $\pm$ 1.6	3	0.42	5.1
5SD+LD	Reverted	Leaf (69-77)	2	63.7	113.1	27.7 $\pm$ 4.2	3	0.44	5.6

<sup>a</sup> Geometric means presented; analysis of variance on log transformed data n.s.

<sup>b</sup> Analysis of variance n.s.



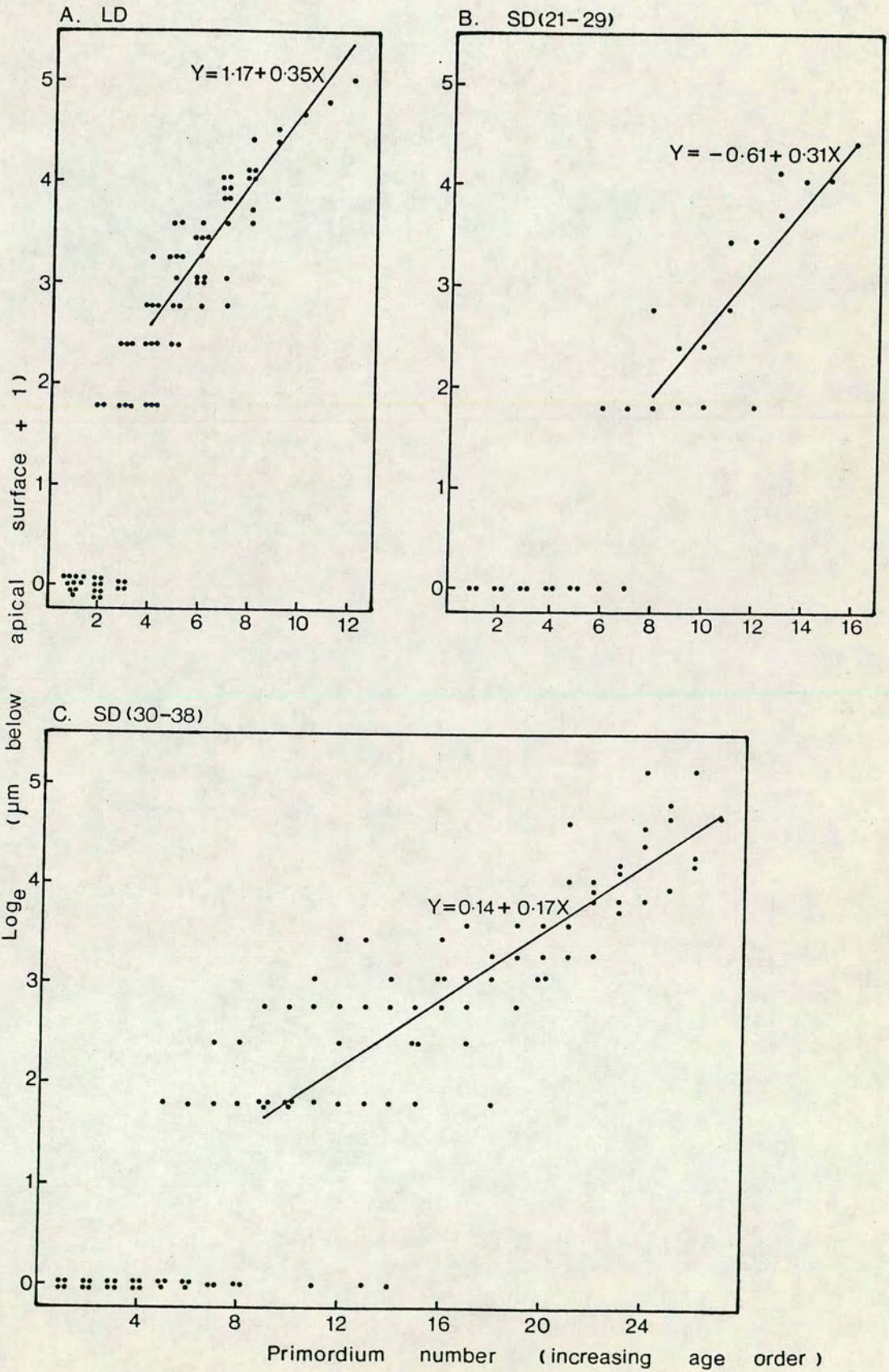
$\underline{r}$  is the plastochron ratio, by analogy the length of the frustum on initiation is  $\log_e \underline{v}$ , where  $\underline{v}$  is the relative vertical distance of successive primordia from the apical surface. As Richards (1951) says of the plastochron ratio, "one may define how one pleases the size of the circle to represent the mean area of the apex, but once this is defined the 'primordial area' is automatically defined also .... In the present case the area taken to represent the 'bare' apex at its mean size is that circle on whose circumference the geometrical centres of the primordia make their first appearance" (my emphasis). Similarly, I define the frustum as being initiated when it can first be measured (i.e. between the first and second primordia below the apical surface), equivalent to defining the primordium as being initiated at the time it can first be seen. Just as the tissue generating the primordium is the tissue nearer the centre of the apex than the first primordium, the tissue generating the frustum is the tissue distal to the first frustum. The length of the tissue distal to the first frustum has already been defined as  $\gg \log_e (5 \mu\text{m} + 1)$  (see p. 24), because this is the level at which a primordium is defined as first being below the apical surface. The procedure used to estimate the length of the frustum on initiation (relative to the tissue generating it) is illustrated in Fig. 17.

The length of the frustum on initiation relative to the length of the tissue distal to it, measured in this way, decreased in the flower compared to the vegetative apex, and was also smaller in the reverted apex (Table 18).



Figure 17. Estimation of the length of the frustum on initiation in vegetative (LD), flowering (SD), and reverting (5SD+LD) plants at different developmental stages.  $\log_e(\mu\text{m below the apical surface} + 1)$  of the insertion points of primordia from plants growing (A) vegetatively in LD, (B) and (C) flowering in SD at successive developmental stages, (D) and (E) reverting in 5SD+LD at successive developmental stages, are plotted as a function of primordium number (the youngest primordium visible in the transverse section grazing the apical surface = P1), in each case. (Numbers in brackets are the total number of leaves + primordia at each developmental stage in SD and 5SD+LD). In LD the mean transformed distances of P1-P3 below the apical surface are  $< \log_e(5\mu\text{m} + 1)$ ; to allow comparison with flowering and reverting apices these primordia are defined as being on the apical surface. Regression lines were fitted for primordia below the apical surface in each treatment stage; the regression coefficients estimate  $\log_e \underline{v}$  where  $\underline{v}$  is the relative distance of any 2 successive primordia from the apical surface.  $\log_e \underline{v}$  is an estimate of the length of the frustum on initiation, relative to the tissue distal to the first primordium below the apical surface.







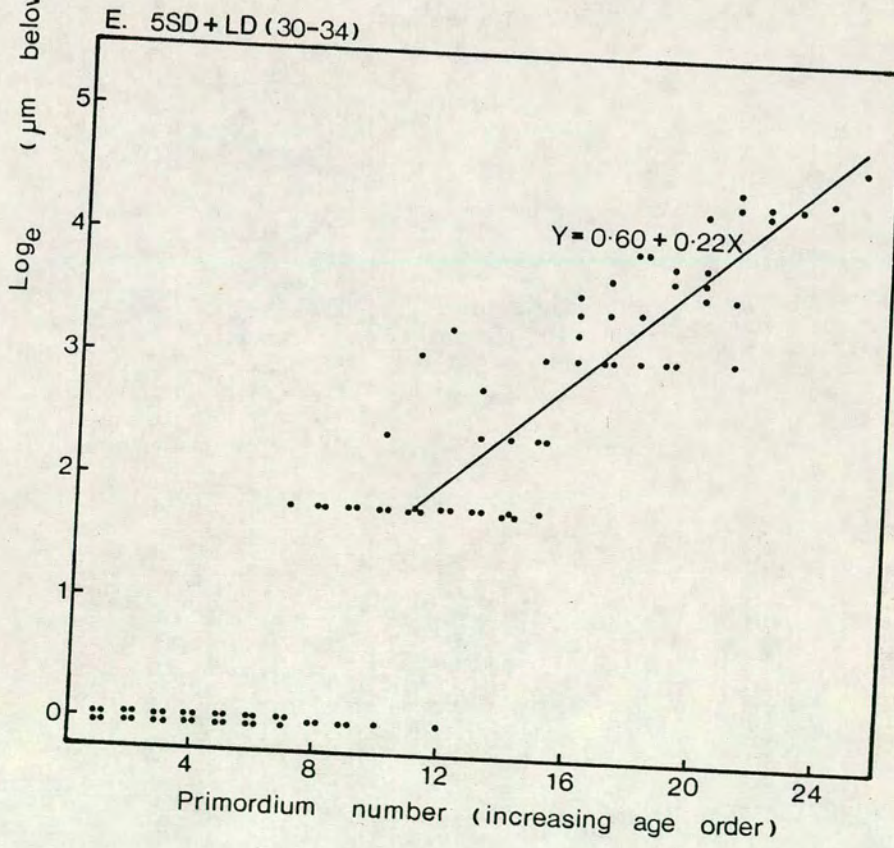
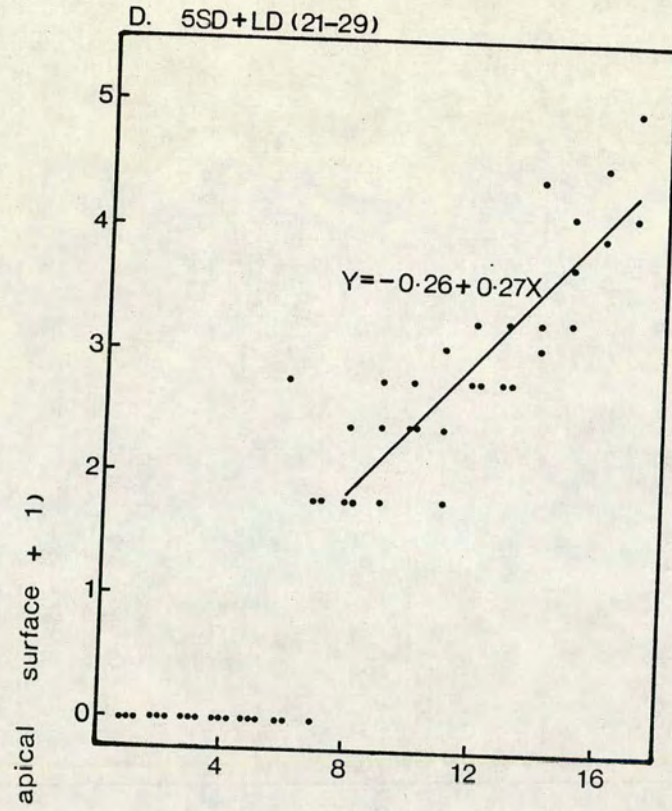




TABLE 18. Lengths of stem frusta on initiation during different phases of development of vegetative (LD), flowering (SD), and reverting (5SD+LD) plants sampled between days 0 and 12 after the beginning of experimental treatments

Treatment	Type of apex	Stage of development (total no. of leaves + primordia)	Number of plants per sample	Mean distance of the first primordium below apical surface from apical surface (length of the tissue generating the frustum) ( $\mu\text{m}$ ) (1) <sup>a</sup>	Length of frustum at initiation relative to the length of tissue generating the frustum ( $\log_e v$ ) ( $\pm 1$ s.e.) (2)	Absolute length of the frustum at initiation (1) x (2) ( $\mu\text{m}$ )
LD	Vegetative	Leaf	10	10.2	$0.35 \pm 0.02^b$	3.6
SD	Flowering	Mid-petal (21-29)	2	8.8	$0.31 \pm 0.06$	2.7
		Late petal (30-38)	4	6.7	$0.17 \pm 0.01^c$	1.1
5SD+LD	Reverting	Intermediate part/leaf (21-29)	3	6.3	$0.27 \pm 0.02$	1.7
		Leaf (30-34)	4	7.2	$0.22 \pm 0.02^c$	1.6

<sup>a</sup> Geometric means presented; analysis of variance on log transformed data n.s.

<sup>b</sup> Significantly different from c at  $P=0.001$ .



The tissue distal to the first frustum was 6-11  $\mu\text{m}$  long; the absolute length of the frustum on initiation, calculated as the product of this and the relative length, was also lower in the flowering and reverted plants than in the vegetative plants (Table 18). The larger frustum on initiation in the vegetative plants, compared with the flowering plants, is correlated with the length of internodes at maturity: vegetative plants have internodes separating their leaves, whilst flowering plants have no visible internodes separating the parts of the mature flower. Reverted plants at the Leaf/ stage in the 5SD + LD treatment had initiated 30-34 parts, 10 of which were on the apical surface (Table 15). Therefore, estimates of frustum length at initiation, in plants at this stage, were made from parts 10-24. All these parts lay within the reversion zone of mature R4 plants, since the first internode above this zone occurred above part number 24.5 (1 s.e. = 0.7, n = 39). Consequently, the similar values for frustum length at initiation (relative or absolute) in SD and 5SD + LD are consistent with the hypothesis that a smaller frustum length at initiation may result in a lack of internode development at maturity.

On reversion, then, at the time that intermediate parts and the first leaves were initiated, the apex initiated frusta of the same size as those found in the normal flower of plants in SD. The formation of intermediate parts and leaves was therefore not accompanied by reversion of the apex to the vertical growth pattern found in vegetative (LD) plants. In the next section



further measurements, designed to determine whether such a change eventually occurs to give rise to the internodes seen after the reversion zone, are presented.

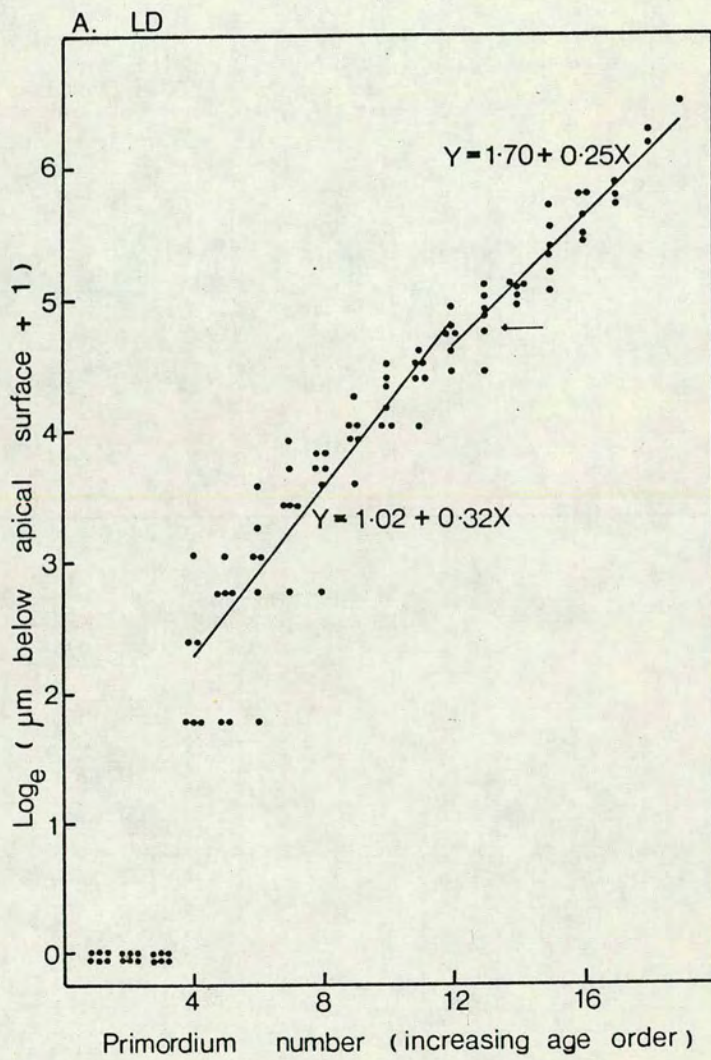
B. Later development of the flowering and reverted apices  
(days 11 - 21)

During this later phase of development apices had initiated more primordia than during the earlier phase, in all three treatments (Fig. 10). This meant that in this case more primordia were available for estimation of frustum length at initiation (Fig. 18A, B and C), and measurements could be made up to about 500  $\mu\text{m}$  below the apical surface, whereas during the earlier phase of development only primordium distances up to about 150  $\mu\text{m}$  below the apical surface were available (Fig. 17A, B and C). To allow comparison between the earlier and later phases of development in LD, frustum length at initiation was estimated, as before, from the regression coefficient of the line fitted to the  $\log_e (\mu\text{m} + 1)$  transformed distances of P4-P12 below the apical surface (Fig. 18A). In SD and 5SD + LD the most important factor was to estimate frustum length at initiation using primordium distances below the apical surface over the equivalent part of the apex to that used in LD, i.e. the part next to primordia with mean transformed distances below the apical surface less than that of P12 in LD (vegetative) controls. Since this distance was equivalent to 111.2  $\mu\text{m}$  (see Fig. 18A), in SD and 5SD + LD frustum length at initiation was estimated from those primordia with mean transformed distance

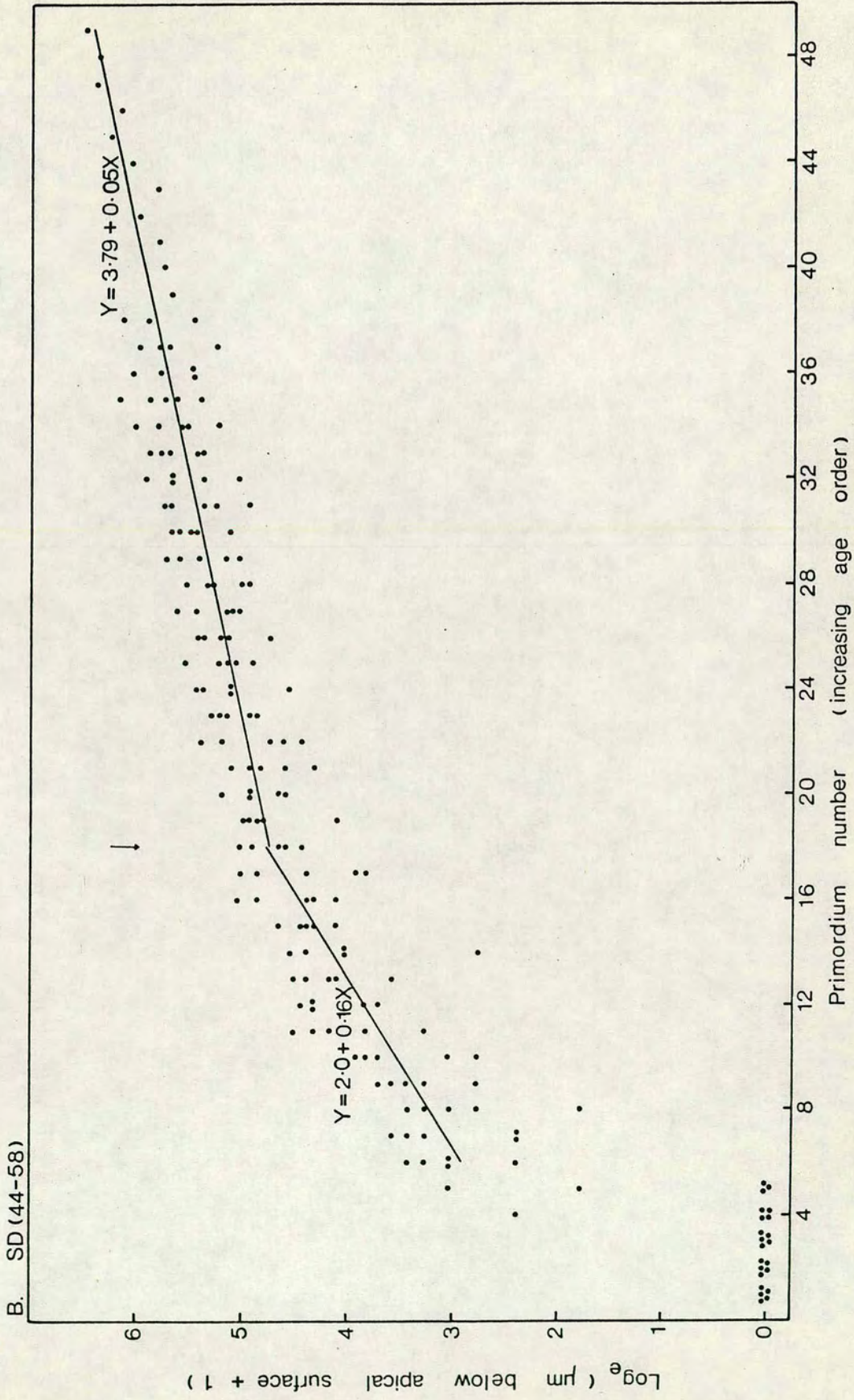


Figure 18. Estimation of the length of the frustum on initiation in (A) vegetative (LD), (B) flowering (SD), and (C) reverted (5SD+LD) plants at stages of development subsequent to those illustrated in Fig. 17.  $\log_e (\mu\text{m below the apical surface} + 1)$  of the insertion points of primordia are plotted as a function of primordium number in each case. See legend to Fig. 17 for details of method of estimation of frustum length at initiation.  $\log_e \underline{v}$  for frusta proximal to P12 in vegetative (LD) plants is estimated by the regression line fitted to primordia P12-P19. To allow comparison with this, in flowering and reverted apices regression lines were fitted to primordia proximal to the mean transformed distance of P12 in LD,  $\log_e (111.2 \mu\text{m} + 1)$  (see arrows); the regression coefficients again estimate  $\log_e \underline{v}$  (see also Table 22).

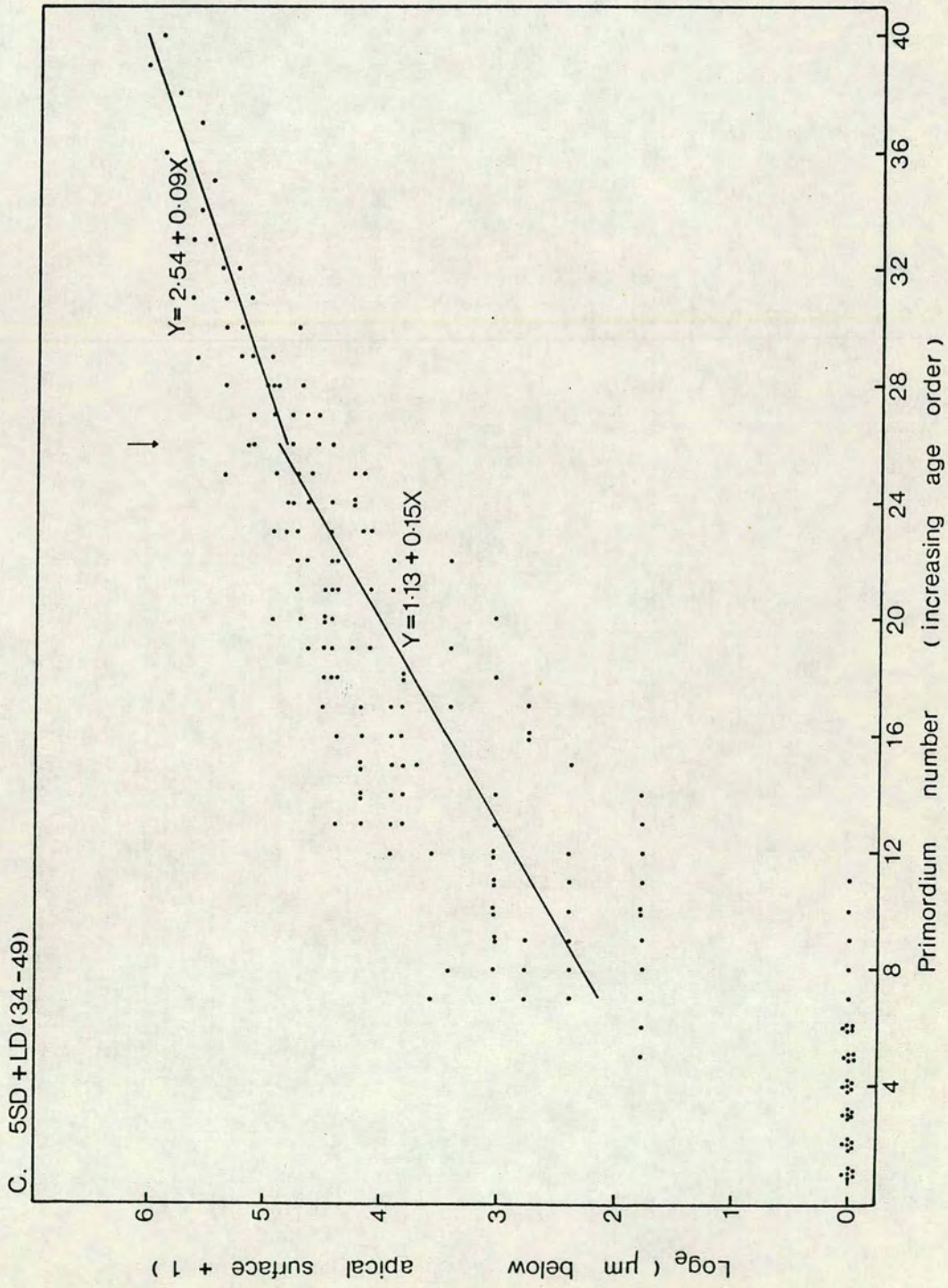














equivalent to  $\leq 111.2 \mu\text{m}$  below the apical surface (Fig. 18B and C). Examination of Fig. 18A, B and C indicates that this procedure is appropriate, since prior to a distance equivalent to  $111.2 \mu\text{m}$  below the apical surface are displaced from the apical surface at a lower rate than those less than this distance away, in all three treatments.

In flowering (SD) and reverted (5SD + LD) plants frustum length at initiation, relative to the length of the tissue generating the frusta, measured in this way, was smaller than in vegetative (LD) plants (Table 19). However, in SD and 5SD + LD frustum length at initiation was approximately the same, even though in the mature flower internodes were absent, whilst they were present in the mature reverted plant (see Table 11, p.72 ).

The absolute length of the frustum/<sup>at initiation</sup> was about the same in vegetative and flowering plants, and was markedly smaller in reverted plants (Table 19). This was not true earlier in development, when flowering and reverted plants initiated frusta of very similar absolute length (see Table 18), and was due to the fact that in flowering plants the length of the tissue generating the frusta was about twice that in vegetative and reverted plants (Table 19). This may be because some of the plants in SD had, by this stage of development, begun to initiate, or initiated, carpels (see Fig. 10, p.75 ), and so ceased to initiate new frusta. After this had occurred, the tissue which previously generated the new frusta presumably began to differentiate and elongate. As a result it would have



TABLE 19. Lengths of stem frusta on initiation in vegetative (LD), flowering (SD), and reverted (5SD+LD) plants sampled between days 11 and 21 after the beginning of experimental treatments

Treatment	Type of apex	Stage of development (total no. leaves + primordia)	Number of plants per sample	Mean distance of the first primordium below apical surface from the apical surface (length of the tissue generating the frustum) ( $\mu\text{m}$ ) <sup>a</sup> (1)	Length of the frustum at initiation relative to the length of tissue generating the frustum ( $\log_e \bar{y}$ ) ( $\pm 1$ s.e.) (2)	Absolute length of the frustum at initiation (1) x (2) ( $\mu\text{m}$ )
LD	Vegetative	Leaf	6	9.1	$0.32 \pm 0.02^b$	2.9
SD	Flowering	Stamen/carpel (44-58)	5	20.8	$0.16 \pm 0.02^c$	3.3
5SD+LD	Reverting	Leaf (34-49)	6	9.6	$0.15 \pm 0.01^c$	1.4

a Geometric means presented; analysis of variance on log transformed data ns.

b Significantly different from c at  $P=0.01$ .



become much longer in flowering plants than in vegetative and reverted plants, which continued to initiate new frusta.

Therefore, internode length at maturity was not correlated with frustum length at initiation: reverted apices developed long internodes after reversion, but initiated frusta of about the same length (relative to the length of the tissue generating the frusta) as flowering apices.

C. The mature reverted (5SD + LD) and vegetative (LD) apices (days 70-74)

In reverted (5SD + LD) apices sampled about 10 weeks after the beginning of experimental treatments the length of the frustum on initiation, relative to the length of the tissue generating the frusta, and in absolute terms, was very similar to that in vegetative (LD) apices sampled between days 0 and 12 (Fig. 19B; compare Tables 18 and 20). This indicates that, as in the case of the radial growth parameters (Section 4.2.3, p.86 ), the reverted apex eventually returned to a mode of growth typical of vegetative (LD) plants sampled between days 0 and 12. Vegetative apices sampled about 10 weeks after the beginning of experimental treatments initiated frusta that were slightly smaller than at earlier stages of development (Fig. 19A; compare Tables 18 and 20), so that the reverted apex was more similar in this respect to the original vegetative apex than to the vegetative apex of its own chronological age (Fig. 19; Table 20).



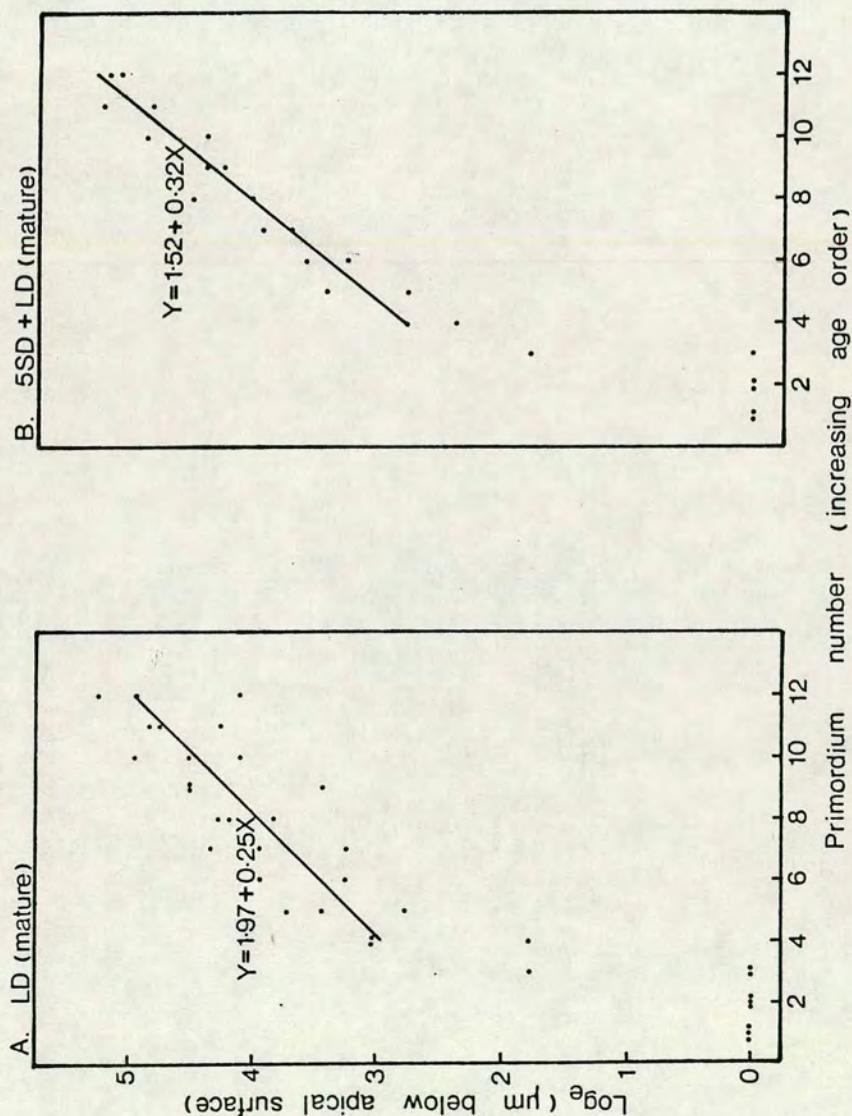


Figure 19. Estimation of the length of the frustum on initiation in (A) vegetative (LD) and (B) reverted (5SD+LD) plants sampled 70-74 days after the beginning of experimental treatments.  $\text{Log}_e$  ( $\mu\text{m}$  below the apical surface + 1) of the insertion points of primordia P1-P12 are plotted as a function of primordium number. Regression lines are fitted to primordia below the apical surface (P4-P12), and the regression coefficients estimate  $\log_e v$ , the length of the frustum on initiation, relative to the tissue distal to the first primordium below the apical surface (see Legend to Fig. 17 for further details of method).



TABLE 20. Lengths of stem frusta on initiation in vegetative (LD) and reverted (R4) (5SD+LD) plants sampled 70-74 days after the beginning of experimental treatments

Treatment	Type of apex	Stage of development (total no. leaves + primordia)	Number of plants per sample	Mean distance of the first primordium below the apical surface from the apical surface (length of the tissue generating the frustum) (μm) (1)	Length of the frustum at initiation relative to the length of the tissue generating the frustum (log <sub>e</sub> v) ( $\pm$ 1 s.e.)(2)	Absolute length of the frustum at initiation (1) x (2) (μm)
LD	Vegetative	Leaf	3	12.8	0.25 $\pm$ 0.02 <sup>a</sup>	3.2
5SD+LD	Reverted	Leaf (69-77)	2	12.3	0.32 $\pm$ 0.02 <sup>b</sup>	3.9

a significantly different from b at P=0.05.



#### 4.2.5 Area and vertical relative growth rates per day of the apex

##### A. Early flower development and reversion (days 0 - 12)

At the time of petal initiation both the area and the vertical relative growth rates per day of the apex had increased about two- to three-fold (Table 21). In the reverting apex the area and vertical relative growth rates per day were intermediate between the rates in apices those in vegetative/and/flowering apices at the Mid-petal stage. The higher growth rate was therefore specific to petal initiation, since it did not occur during initiation of parts of the same plastochron age in the reverting apex. Even so, the reverted apex grew at a higher rate than the vegetative (LD) apex, indicating that reversion to leaf initiation did not involve complete reversal of the increase in growth rate that accompanied flowering.

##### B. Later development of the flowering and reverted apices (days 11 - 21)

The area relative growth rate per day was about the same at this later stage of development as during the earlier stages, in all three treatments (compare Tables 21 and 22). The flowering apices grew about twice as fast as the reverted apices, which grew slightly faster than the vegetative (LD) apices. These differences between the three treatments were therefore not transitory; nor, in the case of the flowering and reverting apices, was a particular area relative growth rate associated with the formation of organs of a particular type.



TABLE 21. Area and vertical relative growth rates per day of the apical surface and frusta during different phases of development of vegetative (LD), flowering (SD), and reverting (5SD+LD) plants sampled between days 0 and 12 after the beginning of experimental treatments

Treatment	Type of apex	Stage of development (total no. of leaves + primordia)	Area relative growth rate day <sup>-1</sup> (2log <sub>e</sub> r x rate of prim <sub>ord</sub> ium initiation) <sup>a</sup>	Vertical relative growth rate day <sup>-1</sup> (log <sub>e</sub> v x rate of prim <sub>ord</sub> ium initiation) <sup>a</sup>
LD	Vegetative	Leaf	0.37	0.32
SD	Flowering	Mid petal (21-29)	0.87	1.18
		Late petal (30-38)	0.99	0.65
5SD+LD	Reverting	Intermediate part/leaf (21-29)	0.50	0.68
		Leaf (30-34)	0.50	0.55

<sup>a</sup> Since both area and vertical relative growth rates day<sup>-1</sup> are derived from values (see Tables 12, 15 and 18) which have error terms, standard errors cannot be calculated for the values presented.



TABLE 22. Area and vertical relative growth rates per day of the apical surface and frusta during different phases of development of vegetative (LD), flowering (SD), and reverted (5SD+LD) apices sampled between days 11 and 21 after the beginning of experimental treatments

Treatment	Type of apex	Stage of development (total no. leaves + primordia)	Area relative growth rate day <sup>-1</sup> (2log <sub>e</sub> r x rate of primordium <sup>a</sup> initiation)	Vertical relative growth rate day <sup>-1</sup> (log <sub>e</sub> v x rate of primordium initiation) <sup>a</sup> log <sub>e</sub> v from frusta of mean transformed distance
				$\leq \log_e (111.2\mu\text{m}+1)$ below apical surface $> \log_e (111.2\mu\text{m}+1)$ below apical surface
LD	Vegetative	Leaf	0.41	0.29
SD	Flowering	Stamen/carpel (44-58) <sup>b</sup>	1.09	0.54
5SD+LD	Reverted	Leaf (34-49)	0.51	0.33
				0.19

<sup>a</sup> Since both area and vertical growth rates day<sup>-1</sup> are derived from values (see Tables 15, 18 and 19) which have error terms, standard errors cannot be calculated for the values presented.

<sup>b</sup> plants at the Stamen/carpel stage in SD had all initiated at least 44 leaves + primordia on sampling. The average rate of primordium initiation for plants with more than about 43 leaves + primordia is about 3.4 primordia day<sup>-1</sup> (see Fig. 10, p. 75; Table 12, p. 76). Therefore this value was used to calculate area and vertical relative growth rates day<sup>-1</sup> for plants at this developmental stage in SD.



In flowering apices at the Stamen/carpel stage the vertical relative growth rate per day of frusta of mean transformed distance,  $\log_e (\mu\text{m} + 1)$ ,  $\leq$   $\log_e (111.2 \mu\text{m} + 1)$  below the apical surface remained about twice that in vegetative (LD) apices (Table 22). However, in reverted apices it was much more similar to that in vegetative apices than it was at earlier stages of development (compare Table 21 and 22), suggesting that very soon after the reverted apex began to initiate leaves again the vertical relative growth rate per day may have returned to its original vegetative (LD) value. The vertical relative growth rate per day of the older frusta (mean transformed distance,  $\log_e (\mu\text{m} + 1)$ , greater than  $\log_e (111.2 \mu\text{m} + 1)$  below the apical surface) was very similar in the 3 treatments (Table 22). This was true, even though, at maturity, vegetative plants had frusta which were much longer than those associated with the intermediate parts of reverted plants, which were in turn longer than those associated with the petals of normally flowering plants (Table 23).

Frustum length at maturity was, therefore, not correlated with the vertical relative growth rate per day of either the younger or the older frusta; nor was it correlated with frustum length at initiation (see Section 4.2.4 B, p.102). It was, however, correlated with the rate of vertical growth per plastochron of the older frusta, as measured by the regression coefficients of the <sup>regression</sup> lines fitted in Fig. 18A, B and C.



TABLE 23. Lengths of stem frusta (node + internode below) at maturity in vegetative (LD), flowering (SD), and reverted plants (R4) (5SD+LD). Data for flowering and reverted plants were obtained from red-flowered plants only; measurements were made when the terminal flower of plants in SD was fully opened (42-49 days after the beginning of experimental treatments). LD plants were measured 70 days after the beginning of experimental treatments, at which time the measured frusta were assumed to have stopped growing

Treatment		LD					SD		5SD+LD
Part number and type at which frustum length measured	Type	Leaves					Petals		Intermediate parts
	Number	11	12	13	14	15	First petal- last petal		Parts subtending internodes < 1 mm long
Length of stem frustum (node + internode below) (mm) (+1 s.e.)		16+4	10+3	18+3	12+1	10+3	0.18+0.01		0.47+0.07
Number of replicates		3					8		5



C. The mature reverted (5SD + LD) and vegetative (LD) apices (days 70-74)

By this stage of development vegetative and reverted apices had very similar area and vertical growth rates per day (Table 24). These were markedly lower than at earlier stages of development of vegetative apices (compare Tables 21, 22 and 24), suggesting that the growth rate per day of the shoot apex fell as the plants grew larger and older. The similarity of the growth rates in the two treatments does, however, indicate that reverted plants had, by this time, returned completely to the mode of growth typical of vegetative (LD) plants.



TABLE 24. Area and vertical relative growth rates per day of the apical surface and frusta in vegetative (LD) and reverted (R4) apices (5SD+LD) sampled 70-74 days after the beginning of experimental treatments

Treatment	Type of apex	Stage of development (total no. of leaves + primordia)	Area relative growth rate day <sup>-1</sup> (2log <sub>e</sub> r x rate of primordium initiation) <sup>a</sup>	Vertical relative growth rate day <sup>-1</sup> (log <sub>e</sub> v <sup>b</sup> x rate of primordium initiation) <sup>a</sup>
LD	Vegetative	Leaf	0.28	0.17
5SD+LD	Reverted	Leaf (69-77)	0.24	0.18

a Since both area and vertical growth rates day<sup>-1</sup> are derived from values (see Tables 13, 16 and 21) which have error terms, standard errors cannot be calculated for the values presented. Rates of primordium initiation are those estimated in the two treatments between days 42 and 74 following the beginning of experimental treatments (see Table 13).

b log<sub>e</sub>v from frusta at P4-P12.



## CHAPTER 5: FLOWERING OF THE REVERTED MERISTEM ON RETURN TO SD

In the preceding chapter the changes in morphology, apical growth, and phyllotaxis that took place on flowering in SD and reversion in 5SD + LD were described. It was found that, on reversion, the apex resumed leaf initiation, but the primordia and stem frusta initiated were the same size as those initiated by the flowering apex. Primordium arrangement remained whorled or pseudo-whorled, as in the flowering apex, and it was not until about 15 days after the resumption of leaf initiation, during which time about 30 leaf primordia had been initiated (Section 4.2.1, p. 77), that the reverted apex began to return to the growth pattern typical of the original vegetative (LD) apex.

This work raised the following question: during this 15 day period, do reverted (5SD + LD) plants have the same response to SD as vegetative (LD) plants? The similar growth and phyllotaxis in reverted and flowering apices suggested that reverted plants might be partially evoked; hence their response to SD might be expected to differ from that of vegetative plants. To test if this were so, reverted plants were returned to SD after 5SD + 9LD, and their flowering response analysed. This chapter describes this response. The development of plants left in LD after 5SD + 9LD provided one control; the other control was the response to SD of vegetative (LD) apices of equivalent physiological age to those replaced in SD after 5SD + 9LD. It was assumed that the



total number of leaves plus primordia initiated at the time of transfer to SD gave the best indication of physiological age. Therefore, in a single experiment vegetative plants that had initiated about the same number of leaves plus primordia as reverted plants had initiated after 5SD + 9LD (value calculated from regression = 32.9 (1 s.e. = 1.2); see Fig. 20) were transferred to SD and their flowering response analysed. The control in this experiment was provided by plants remaining vegetative in LD.

In the experiments to be described the reversion response was more uniform than that obtained previously. This was achieved by using only red-flowered plants (see Sections 2.5 and 3.3.2). It will be necessary to compare the results obtained using this technique with the results obtained previously using all flower colours (see Chapter 4). Therefore, it is important to know whether red-flowered plants showed the same response to SD and 5SD + LD as that described in Chapter 4 for all flower colours.

In morphological terms, red flowers and reverted (R4) flowers were very similar to flowers and reverted (R4) flowers of all colours (Table 25). Normal red flowers differed from normal flowers of all colours in only one respect: in red flowers the modification of venation that occurred at the transition to flowering (see Figs. 9 and 22) was apparent slightly earlier than was found for the combined data for all flower colours. This difference was not, however, apparent in R4 reversions (Table 25). Since this was the only difference between red flowers (or



TABLE 25. Comparison of the characteristic features of terminal flowers (SD) and reversions of the R4 type (SSD+LD), of plants of all flower colours, with those of plants with red flowers. All flower colours: combined data from 10 experiments (see also Table 11). Red-flowered plants: combined data from 6 experiments

Treatment	Mean first part (numbering from base upwards) with the following feature(s) (+ 1 s.e.):								
	No axillary structure	Modified venation	Bract	Petal pigment	Petal	Stamen	Carpel	Without petal pigment (leaf)	Above which internode is $\geq$ 1mm long
SD (all flower colours)	9.0+0.1	9.7+0.2 <sup>a</sup>	11.5+0.1	13.3+0.2	15.1+0.2	34.4+0.8	56.1+1.2	-	-
Number of plants	77	66	74	49	76	78	68		
SD (red flowers only)	8.7+0.2	9.0+0.2 <sup>b</sup>	11.8+0.2	12.9+0.2	14.7+0.2	32.5+1.1	50.8+1.7	-	-
Number of plants	31	31	31	31	31	31	31		
5SD+LD (all flower (R4) colours)	9.1+0.2	10.4+0.2	-	15.5+0.3	-	-	-	25.0+0.6	24.5+0.7
Number of plants	39	39	-	39	-	-	-	38	39
5SD+LD (red flowers (R4) only)	9.5+0.4	10.7+0.4	-	14.6+0.5	-	-	-	25.6+1.3	24.4+0.9
Number of plants	17	17	-	17	-	-	-	17	17

a Significantly different from b at P=0.001.



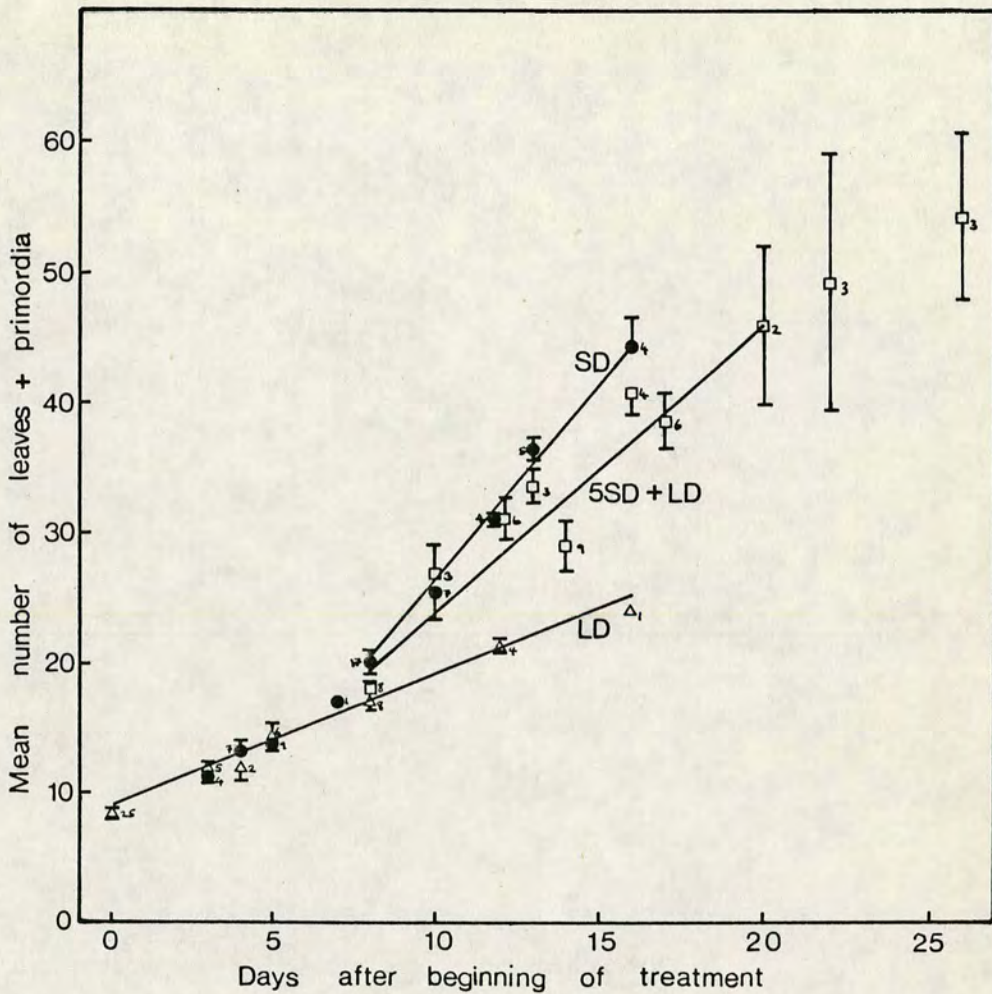


Figure 20. Change of primordium number in vegetative (LD) ( $\Delta$ ), flowering (SD) ( $\bullet$ ), and reverting (5SD+LD) ( $\square$ ), plants between days 0 and 26 following selection. All data are derived from red-flowered plants, except those on day 0, which are derived from plants of all flower colours (plants on day 0 were too small to allow successful decapitation; therefore analysis could not be confined to red-flowered plants at this time). Combined data from five experiments. Mean values + 1 s.e. are presented. The number beside each point indicates the number of replicates in each sample. Full lines are those given by regression equations. Values of the regression coefficients are given in Table 26.



reverted flowers) and all flowers (or reverted flowers) it seems reasonable to conclude that, morphologically, red flowered plants are representative of plants with other flower colours.

When analysis was confined to red-flowered plants, the rates of primordium initiation in SD, 5SD + LD and LD were very similar to the comparable values for plants of all flower colours (compare Fig. 20 with Fig. 10, p.75). Comparison of the regression coefficients of the fitted regression lines in each of these treatments indicated that the rates of primordium initiation were not significantly different in red-flowered plants from the rates of initiation in plants of all flower colours (Table 26). The only marked difference was the relatively steady, though slightly slower rate of initiation in red-flowered plants in SD between days 8 and 16 (Fig. 20); there was no clear evidence for a decrease in the rate of initiation between days 13 and 16 as was found in plants of all flower colours (Fig. 10; Table 26). This may have been because of the more restricted sampling in the data obtained for red-flowered plants.

The sizes of primordia and frusta on initiation estimated for reverted (5SD + LD) plants in the previous chapter (see Tables 15, 16 18 and 19) were very similar to those estimated for reverted (5SD + LD), red-flowered plants in the results to be described (Tables 29 and 31).

It is reasonable to conclude from these comparisons that, whilst showing a more uniform reversion response to 5SD + LD (see Table 9, p.67 ), red-flowered plants showed



TABLE 26.

Comparison of rates of primordium initiation per day of plants of all flower colours growing in LD(vegetative), SD (flowering), and 5SD+LD (reverting), with those of plants with red flowers in the same treatments. Rates of initiation were estimated as the regression coefficients from regression lines fitted to the data of Figure 10 (all flower colours - see also Table 12) and Figure 20 (red-flowered plants)

All flower colours				Red-flowered plant only	
Treatment	Type of apex	Days after beginning of treatment	Rate of initiation of primordia day (+ 1 s.e.)	Days after beginning of treatment	Rate of initiation of primordia day (+ 1 s.e.)
LD	Vegetative	0-21	0.9+0.03 <sup>a</sup>	0-16	1.0+0.04 <sup>b</sup>
		8-13	3.8+0.3 <sup>c</sup>	8-16	3.1+0.1 <sup>d</sup>
SD	Flowering	13-16	1.3+0.2		
		16-20	3.4+0.3		
		8-13	2.5+0.3 <sup>e</sup>	8-20	2.2+0.3 <sup>g</sup>
5SD+LD	Reverting	13-20	2.2+0.3 <sup>f</sup>		

Comparisons a and b; c and d; e, f and g: all n.s.



very similar changes in morphology and rates of primordium initiation on flowering and reversion to those found in plants of all flower colours. The data on the growth pattern and phyllotaxis of reverted plants obtained in the previous chapter are very similar to those obtained for reverted plants in this chapter. This gives further support to the conclusion that valid comparisons can be made between data obtained from red-flowered plants alone, and data obtained from plants of all flower colours.

#### 5.1 Morphological changes on flowering of the reverted meristem

By day 14 reverted plants (5SD + 9LD) had initiated 32 to 33 leaves plus primordia (value calculated from regression = 32.9 (1s.e. = 1.2); see Fig. 20). Reverted (R4) plants began to initiate normal leaves again from about part 26 onwards (Table 25), so that by the time of re-transfer to SD on day 14 about 6-7 leaves (approximately one whorl or pseudo-whorl) had been initiated.

The extent of the effect of re-transfer to SD on the parts of the reverted meristem was best quantified by measurement of the percentage area occupied by petal pigment in successive parts of plants subjected to 5SD + 9LD + SD, and comparing the data with those obtained for plants showing the R4 reversion type after 5SD + LD (Fig. 21A). The reverted plants initiated about 10 to 15 intermediate parts, which had, on average, less than 20 per cent of their area occupied by petal pigment

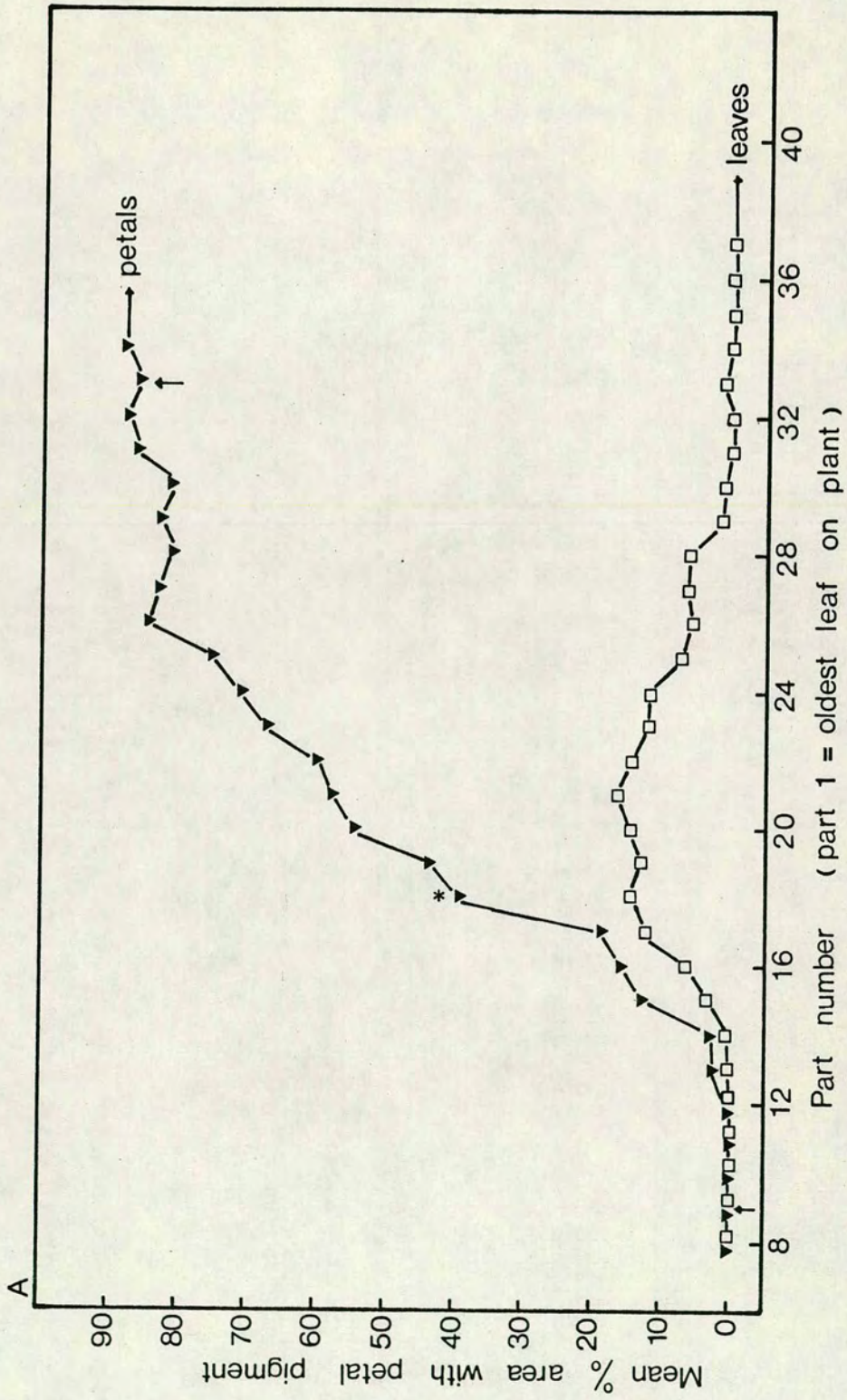


Figure 21. Mean % area with petal pigment in mature parts of reverted (R4) and re-flowering plants.

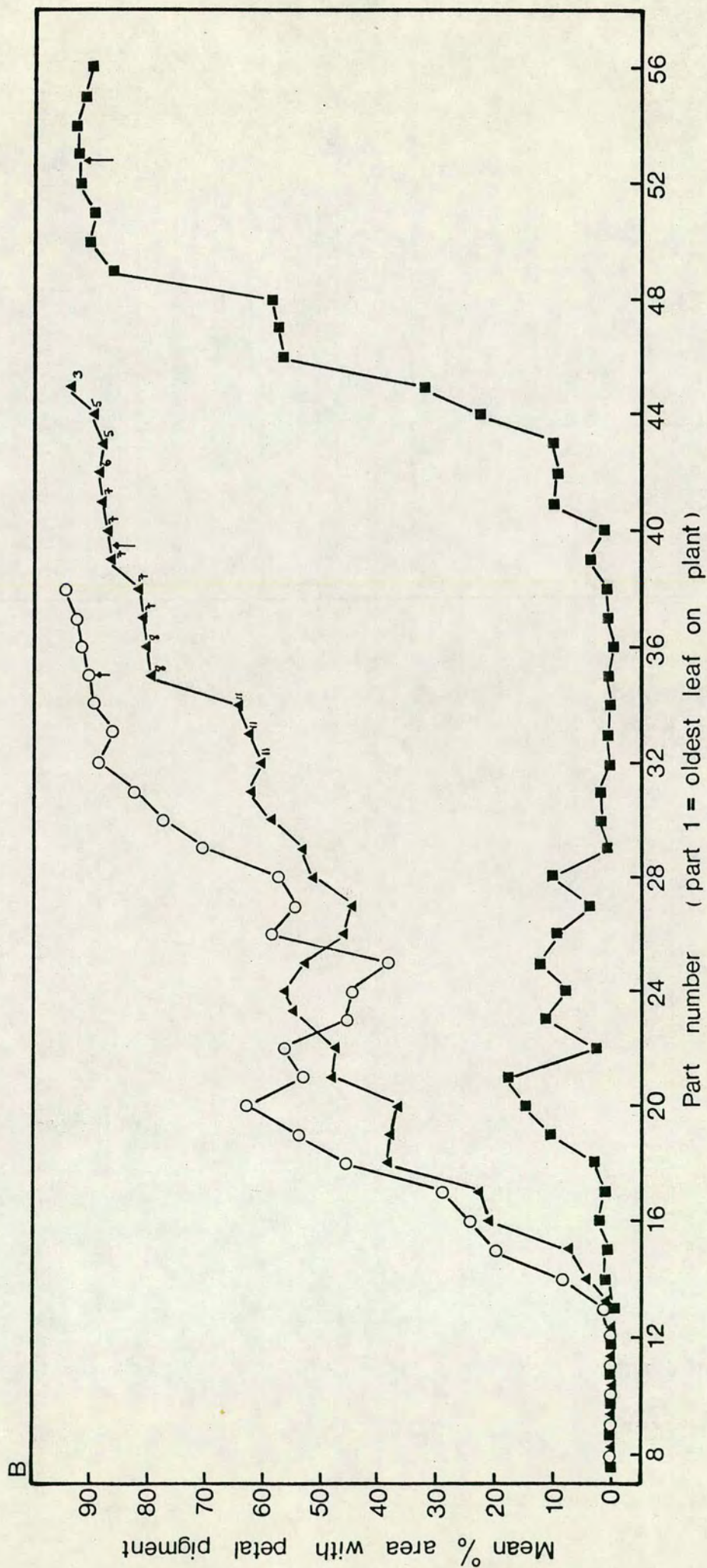
(A) 5SD+LD (□) (n=20); 5SD+9LD+SD (▼) (n=13).

\* 5SD+9LD+SD significantly different from 5SD+LD at P=0.05. (B) 5SD+10LD+SD (○) (n=3); 5SD+12LD+SD (▲) (n=8, except where numbers beside points indicate otherwise); 5SD+20LD+SD<sup>(■)</sup> (n=4). Vertical arrow indicates the predicted mean number of leaves plus primordia at final transfer to SD in each of the five treatments (see also Figure 20). Combined data from red-flowered plants in six experiments.











(Fig. 21A; see also Section 4.1.1, p.69 ). Plants given 5SD + 9LD + SD formed petals (parts without a spur and with more than 50 per cent of their area pigmented - see also Section 4.1.1, p. 69) from part 20 onwards, when this was estimated from the mean percentage area of the surface of each part occupied by petal pigment (Fig. 21A). The first petal was formed, on average, at part 20.7 (1 s.e. = 1.2, n=13), in good agreement with this. Since there were 32 leaves plus primordia present at the time of re-transfer to SD (Fig. 20), about 13 primordia developed as petals, instead of developing as intermediate parts or leaves. Furthermore, in the plants given 5SD + 9LD + SD part 18 was the first to show a statistically significant increase in the percentage of its area occupied by petal pigment, when the angular transformed data were compared with those from plants given 5SD + LD (Fig. 21A). Therefore, 15 parts present on the reverted meristem at the time of re-transfer to SD (parts 18-32) showed an increase in the percentage of their area occupied by petal pigment. This effect of SD on primordia already present contrasts strongly with the lack of such an effect in plants transferred to SD on day 0, in which the first part with petal pigment was formed at about part 13, and the first petal was formed at about part 15 (Table 25). These parts were initiated four and six plastochrons, respectively, after transfer of the plants to SD on day 0 (Fig. 20).

Flowering of the reverted (5SD + 9LD) meristem differed in a further respect from that of the vegetative (LD) meristem. Bracts (parts that were less than 50 mm



long at maturity and which lacked a petiole and/or had a spur) were always formed on transfer of vegetative (LD) plants to SD (see Section 4.1.1, p. 69). They were not formed on re-transfer of the reverted (5SD + 9LD) plants to SD, since petals (parts with a petiole and without a spur) were initiated immediately (Fig. 21A). The appearance of the typical parts of the flower formed in response to 5SD + 9LD + SD is contrasted with that of the parts of reverted (5SD + LD) and normal (SD) flowers in Fig. 22A-C.

Plants given 5SD + 9LD + SD went on to initiate their first stamen at part 46.5 on average (1 s.e. = 1.6, n=13), and their first carpel at part 69.5 on average (1 s.e. = 2.0, n=13).

To summarize: reverted (5SD + LD) plants showed an increase in reactivity to SD, compared with vegetative (LD) plants. Fifteen parts already present at the time of re-transfer to SD showed an increase in the percentage of their surface occupied by petal pigment, in the 5SD + 9LD + SD treatment. Primordia present at the time of transfer of vegetative (LD) plants to SD on day 0 had no petal pigment at maturity. A further difference was that bracts were not formed when reverted (5SD + LD) plants were returned to SD, whereas they were always formed when vegetative plants were transferred to SD on day 0.

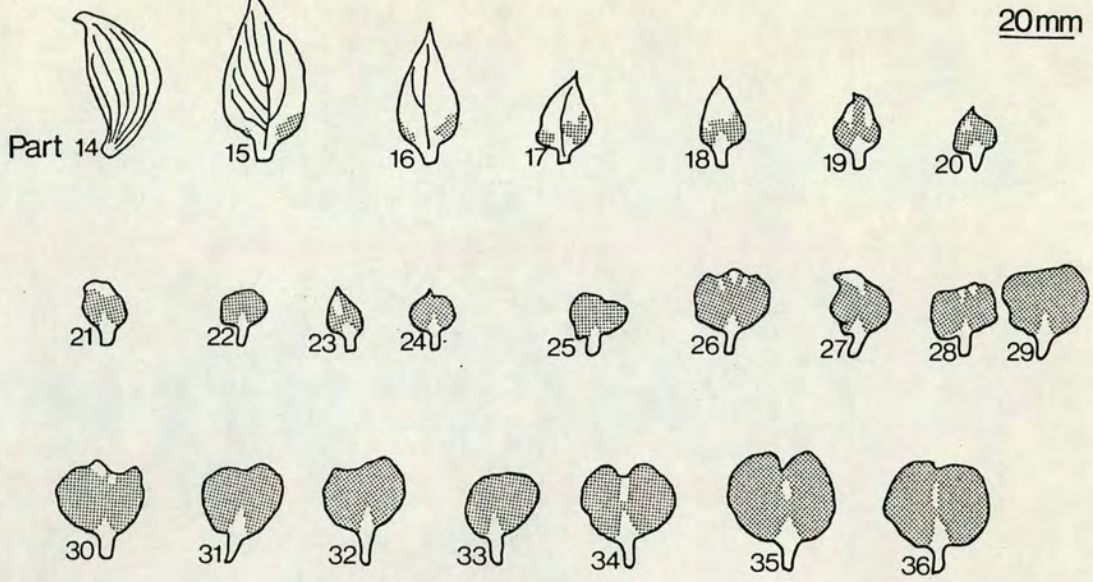
The previous comparisons in this section have been concerned with the differences between flowering of reverted plants, and flowering of vegetative plants trans-



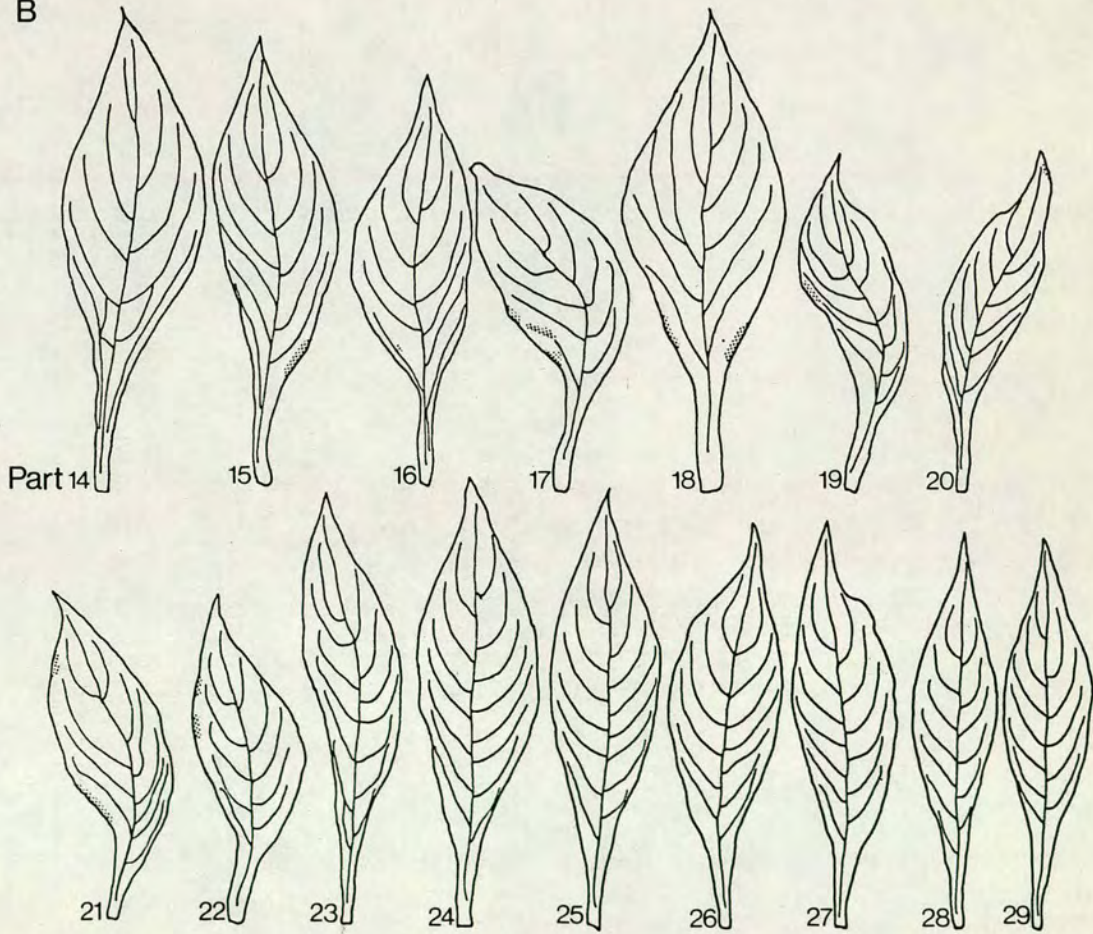
Figure 22. Changes in morphology of successively initiated parts (A) of the flower of re-flowered plants (5SD+9LD+SD) (B) of the reverted (R4) flower (5SD+LD), and of the normal flower of plants transferred to SD on day 0 (C) and day 29 (D) after selection for developmental uniformity on day 0. All the plants were red-flowered. Areas with pigment typical of petals are shaded. (▼) indicates the presence of a spur. Part 1 = the lowest leaf on the plants. Arrows indicate the number of leaves plus primordia initiated at the time of transfer of plants to SD on day 0 (C) and day 29 (D).



A

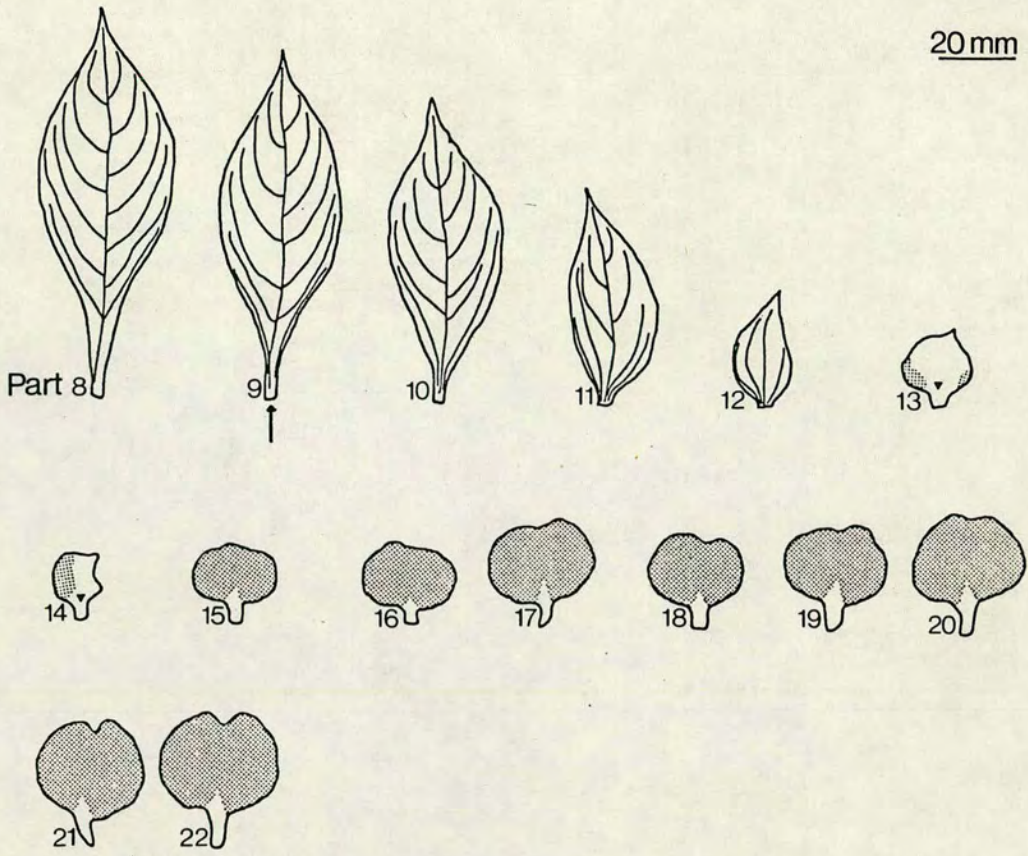


B

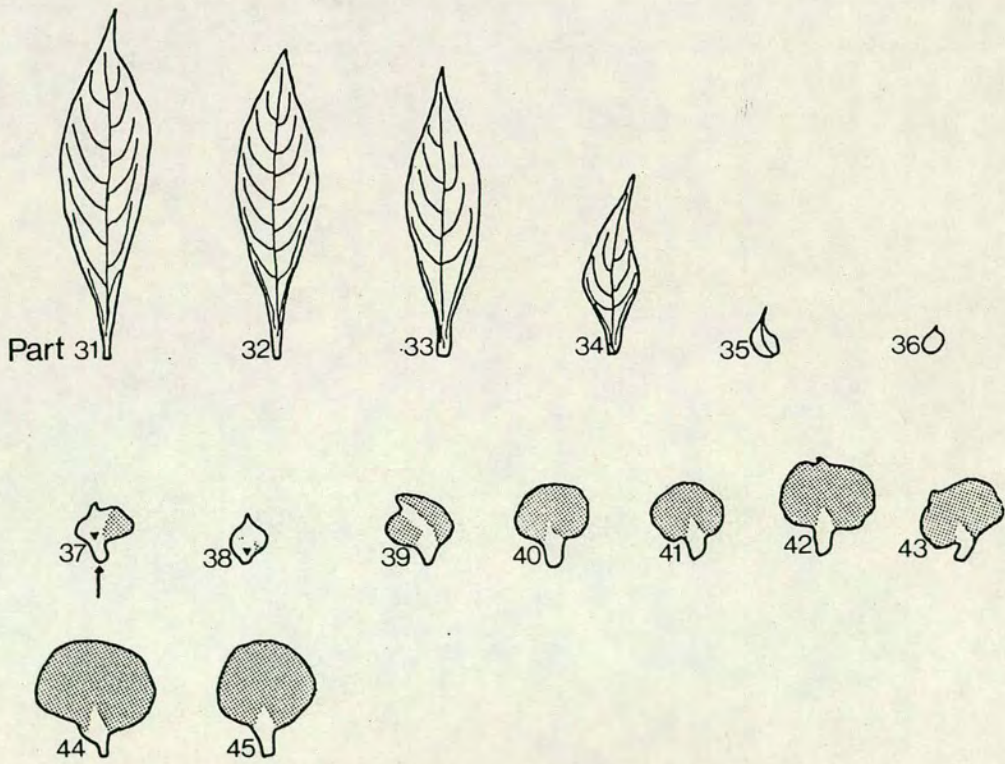




C



D





ferred to SD on day 0. However, reverted plants had initiated about 33 leaves plus primordia on re-transfer to SD after 5SD + LD, whereas vegetative plants had initiated only about 9 leaves plus primordia on transfer to SD on day 0 (Fig. 20). Therefore, a control experiment was set up in which vegetative (LD) plants were transferred to SD on day 29, when they had initiated about the same number of leaves plus primordia as reverted plants on re-transfer to SD. Assuming that the total number of leaves plus primordia initiated accurately reflects the physiological age of a plant, comparison could then be made between flowering of reverted and vegetative plants of equivalent physiological age. The morphology of flowers formed by vegetative (LD) plants transferred to SD on day 29 will now be described.

Thirty six to 37 leaves plus primordia had been initiated by plants remaining vegetative in LD until day 29 (Fig. 23B). This was the number of leaves plus primordia estimated to have been initiated by reverting plants on day 14 (the time of re-transfer to SD) at the time the experiment was designed. Since reverting plants had in fact initiated 32 to 33 leaves plus primordia by day 14 (Fig. 20, p.122), slightly more leaves plus primordia had been initiated by vegetative plants at the time of their transfer to SD on day 29. However, the values were considered to be sufficiently similar to allow comparisons to be made.

In vegetative (LD) plants transferred to SD on day 29, although 36 leaves plus primordia were present at the



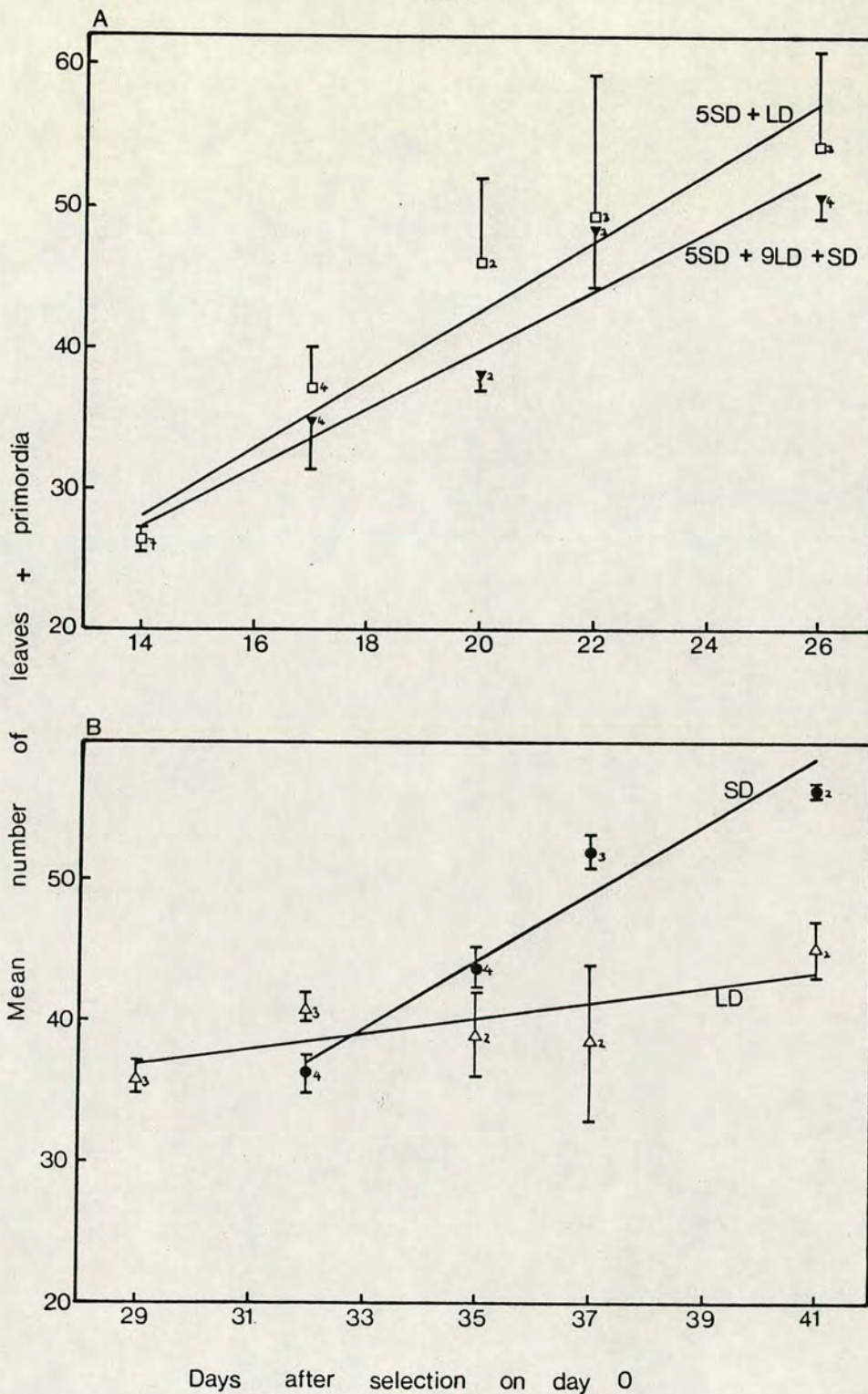


Figure 23. Change of primordium number in red-flowered plants. Mean values  $\pm 1$  s.e. are presented. The number beside each point indicates the number of replicates in each sample. Full lines are those given by regression equations. Values of the regression coefficients are given in Tables 27 and 28. (A) Change of primordium number in reverted plants (5SD+LD) ( $\square$ ), and re-flowering plants (5SD+9LD+SD) ( $\blacktriangledown$ ), between days 14 and 26 after selection for developmental uniformity on day 0, (B) Change of primordium number in vegetative (LD) ( $\Delta$ ), and flowering (SD) ( $\bullet$ ), plants, between days 29 and 42 after selection for developmental uniformity on day 0. Plants to be induced to flower were transferred to SD on day 29.



time of transfer to SD, the first leaf without an axillary structure was part 31.9 (1 s.e. = 2.0, n=8), and the first bract was part 34.4 (1 s.e. = 1.1, n=8). This indicates that parts already present at the time of transfer to SD had their development modified as a result of the transfer; this did not occur when plants were transferred to SD on day 0 (see Section 4.1.1, p.69 ). The first part with petal pigment was part 37.6 (1 s.e. = 1.5, n=8), and the first petal was part 39.5 (1 s.e. = 1.3, n=8). These parts were initiated about one and three plastochrons, respectively, after transfer of the plants to SD on day 29 (Fig. 23B). In plants transferred to SD on day 0 the first part with petal pigment, and the first petal, were initiated four and six plastochrons, respectively, after transfer (p.129). Therefore, although the changes in morphology, and the sequence in which they occurred during the transition from leaf to petal initiation were the same in plants transferred to SD on day 0 and in those transferred on day 29, the position of the parts showing these changes differed in relation to the number of leaves plus primordia present at the time of the transfer. This is illustrated in Fig. 22 C-D. There was a marked increase in the reactivity of vegetative (LD) plants to SD as they aged (from day 0 to day 29). However, the increased reactivity of plants transferred to SD on day 29 was not the same order as that shown by reverted (5SD + 9LD) plants on re-transfer to SD, since, in the latter, parts present at the time of transfer developed as petals, whereas in the former they did not. The



increased reactivity of reverted plants was confirmed by transferring plants back to SD after 5SD + 10LD, 5SD + 12LD and 5SD + 20LD (Fig. 21B). In each case at least seven primordia present at the time of re-transfer to SD developed as petals.

The conclusion from these morphological data is that the reverted meristem flowered in a different way from the vegetative meristem, since parts already present at the time of re-transfer to SD developed as petals, and no bracts were formed on re-flowering. Parts already present at the time of transfer of vegetative (LD) plants to SD did not develop as petals, and bracts were always formed, whether the transfer to SD took place on day 0 or on day 29.

## 5.2 Changes in apical growth and phyllotaxis

### 5.2.1 Rates of primordium initiation

In a single experiment the rates of primordium initiation were compared in plants transferred to SD after 5SD + 9LD and in control plants given

5SD + LD (Fig. 23A). In both treatments regression lines were fitted to the data between days 14 and 26, with no significant deviations from linearity, although in 5SD + LD the rate of initiation may have begun to decrease between days 22 and 26, as was found previously (see Section 4.2.1, p.74 ). There may also have been a similar decrease in initiation rate at this time in the 5SD + 9LD + SD treatment (Fig. 23A). The regression coefficients of the fitted lines were not significantly different (Table 27),



TABLE 27. Rates of primordium initiation in red-flowered plants growing in 5SD+LD (reverted) and 5SD+9LD+SD (re-flowering). Rates of initiation were estimated as the regression coefficients from regression lines fitted to the data of Figure 23A.

Treatment	Type of apex	Days after beginning of treatment	Rate of initiation primordia day <sup>-1</sup> (+ 1 s.e.) <sup>a</sup>
5SD+LD	Reverted	14-26	2.4 $\pm$ 0.3
5SD+9LD+SD	Re-flowering	14-26	2.1 $\pm$ 0.3

<sup>a</sup> Comparison of regression lines : n.s.

TABLE 28. Rates of primordium initiation in vegetative (LD) and flowering (transfer to SD on day 29) plants sampled between days 29 and 41 after selection for developmental uniformity on day 0. (Data obtained from red-flowered plants only) Rates of initiation were estimated as regression coefficients from regression lines fitted to data of Figure 23B.

Treatment	Type of apex	Days after beginning of treatment	Rate of initiation primordia day <sup>-1</sup> (+ 1 s.e.) <sup>a</sup>
LD	Vegetative	29-41	0.6 $\pm$ 0.3
SD	Flowering	35-41	2.4 $\pm$ 0.4

<sup>a</sup> Comparison of regression lines : significantly different at P=0.05.



and indicated that in both treatments the rate of primordium initiation was approximately constant, and was 2.1 to 2.4 primordia per day. There was no indication that, with the onset of flowering, on return to SD after 5SD + 9LD, the rate of primordium initiation increased as it did on flowering of vegetative (LD) plants transferred to SD on day 0 (see Section 4.2.1, p.74 ).

In the control experiment, in which flower formation was followed in plants of equivalent physiological age (see Section 5.1, p.125) to those given 5SD + 9LD, transfer to SD taking place after 29LD, there was an increase in the rate of primordium initiation at about the time petal initiation began (Fig. 23B). During the initiation of petals, flowering (SD) plants initiated primordia at a rate of about 2.4 per day, whilst vegetative (LD) controls initiated primordia at a rate of about 0.6 per day (Table 28). In both treatments the rate of initiation was slower than when measurements were begun on day 0 (compare Tables 12 and 28). However, the difference between the rate of initiation in LD and that in SD was about 4-fold (Table 28); this is about the same as the difference between plants flowering in SD between days 8 and 12 and vegetative (LD) plants between days 0 and 21 (see Table 12, p. 76 ). The absolute rate of primordium initiation in SD, 2.4 primordia per day, was very similar to that in plants given 5SD + 9LD + SD (compare Tables 27 and 28); the difference between the two types of apex was that plants given 5SD + 9LD + SD showed no change in initiation rate compared to 5SD + LD controls, as flowering resumed, whereas plants transferred to SD after 29LD showed a marked increase in initiation rate, compared to LD controls, as flowering took place.



Therefore, an increase in the rate of primordium initiation is not inevitably associated with the switch from leaf to petal initiation; only when vegetative (LD) plants were transferred to SD was it found. The lack of change in initiation rate when reverted (5SD + 9LD) plants were transferred to SD, and the already higher rate (compared to vegetative (LD) plants) at the time of this transfer, suggests that the reverted apex has already undergone any changes in initiation rate that are usually associated with petal formation.

#### 5.2.2 Divergence angles

When reverted plants were transferred back to SD after 5SD + 9LD, new primordia continued to be initiated in whorls or pseudo-whorls of 5-6 primordia, as they were in reverted plants left in LD (Fig. 24A-D). In the control experiment, vegetative (LD) plants sampled between days 29 and 41 had spiral phyllotaxis (Fig. 25A) with a mean divergence angle of  $138.0^{\circ}$  (1 s.e. =  $0.8^{\circ}$ ,  $n=29$ ). After transfer to SD on day 29, the phyllotaxis changed from spiral to whorled or pseudo-whorled (Fig. 25 B-D), so that by the Mid petal stage the divergence angle between the young petal primordia could no longer be estimated, because their order of initiation could not be determined. This change is similar to that found when plants were transferred to SD on day 0 (see Section 4.2.2, p. 80).

As vegetative (LD) plants aged between days 0 and 42 the phyllotaxis remained spiral and the divergence angle remained constant, whereas in reverted (5SD + 9LD)



200  $\mu$ m

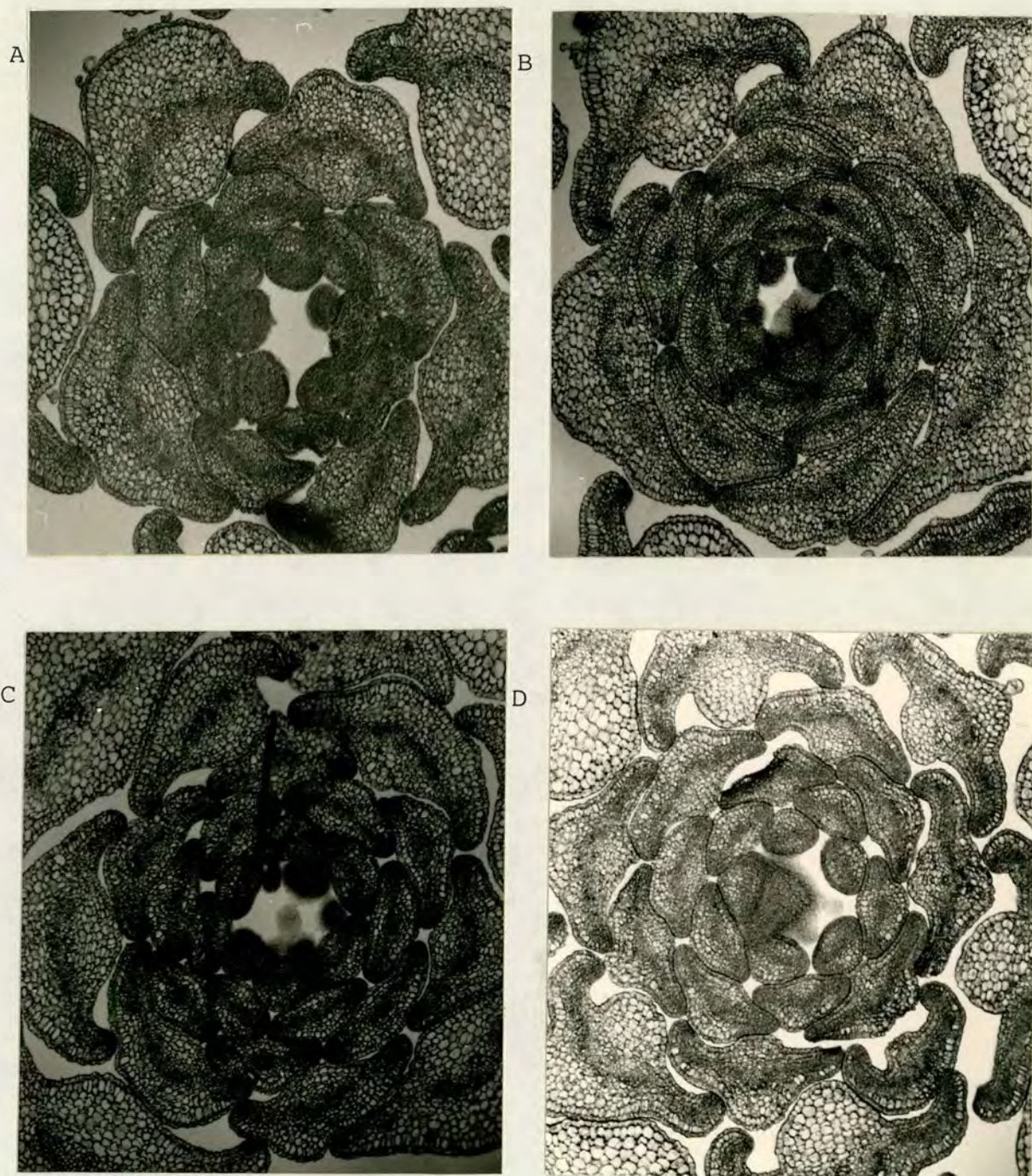


Figure 24. Transverse sections grazing, or just above, the shoot apex of red-flowered plants: re-flowering (5SD+9LD+SD) (A) at the Petal (30-46) stage and (C) at the Stamen (47-69) stage, and reverted (5SD+LD) (B) at the Leaf (30-46) stage and (D) at the Leaf (47-69) stage.



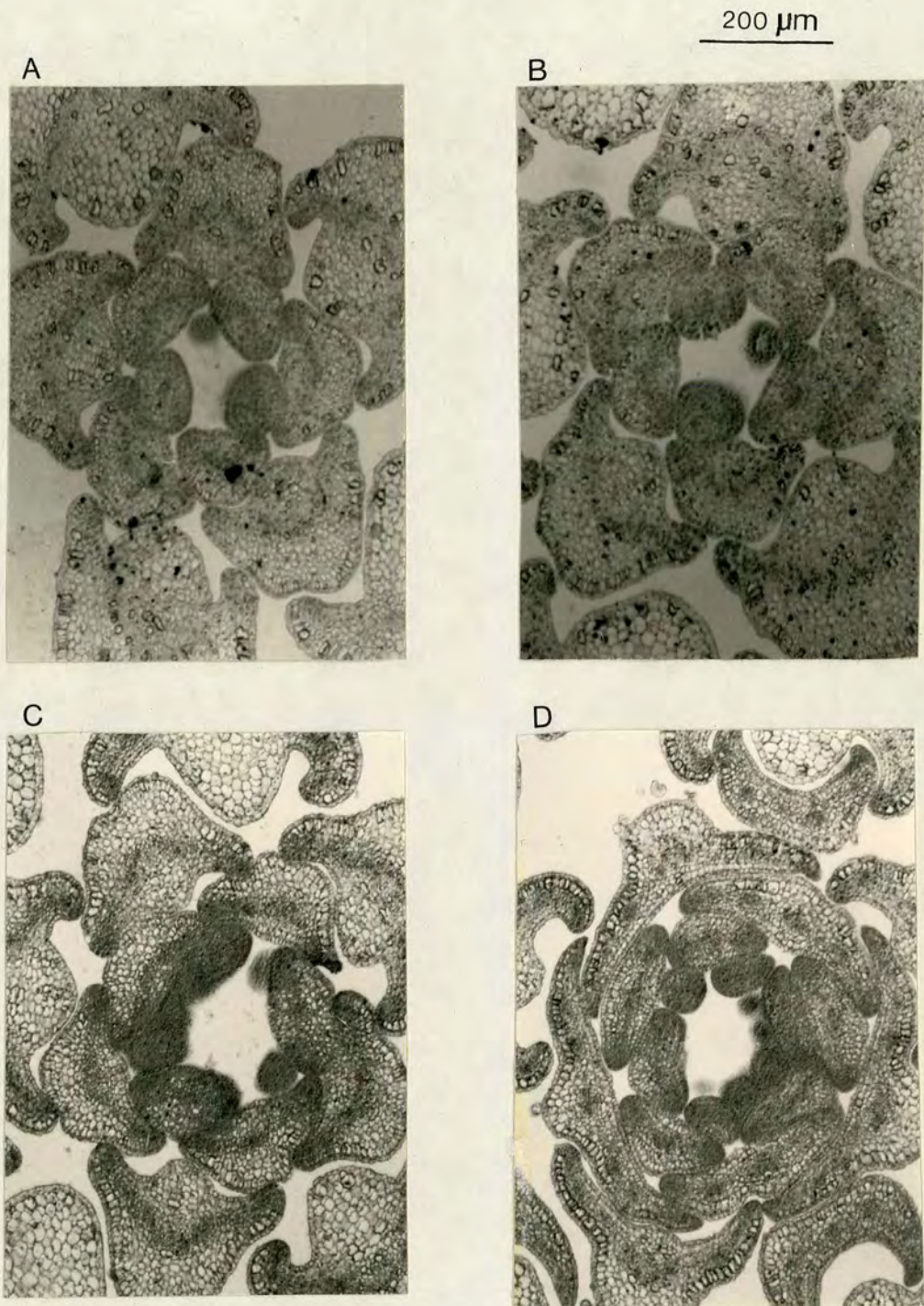


Figure 25. Transverse sections grazing, or just above, the shoot apex of (A) vegetative (LD) plants; and of flowering (SD) plants (B) at the Bract (35-39) stage, (C) at the Early petal (40-49) stage and (D) at the Mid petal (50-59) stage. All plants had red flowers.



apices whorled or pseudo-whorled phyllotaxis had already been attained at the time of re-transfer to SD. Because the phyllotaxis is already whorled or pseudo-whorled in reverted (5SD + 9LD) plants returned to SD, this may in some way facilitate the developmental change in existing primordia from potential leaves to petals.

### 5.2.3 Area of the apical surface and areas of primordia at initiation

In reverted (5SD + LD) plants the data obtained in this experiment (Table 29) confirmed the findings based on plants of all flower colours and described in Section 4.2.3 (see Tables 15 and 16, p.<sup>87</sup>/<sub>93</sub> ). The only difference was that the area of the annulus bearing the primordia on the apical surface decreased significantly between the Intermediate part/leaf (21-29) stage and the Leaf (30-46) stage in this experiment (Table 29), as the number of primordia on the apical surface decreased from 8 to 6, whereas in the previous experiment this decrease in annulus area was non-significant (Table 15, p.87 ). The area of the primordium at initiation (relative to the area of the apical surface and in absolute terms) was about the same in the two experiments. These data confirmed the fact that reverted, leaf-initiating apices did not return immediately to the radial growth pattern of vegetative (LD) apices.

There were no significant differences between the re-flowering (5SD + 9LD + SD) meristem at the Petal (30-46) and Stamen (47-69) stages, and the reverted (5SD + LD)



TABLE 29. Area of the apical surface and areas of primordia on initiation in reverted (5SD+LD) and re-flowering (5SD+9LD+SD) plants (all data obtained from transverse sections of the shoot apex of red-flowered plants)

Treatment	Type of apex	Stage of development (total no. of leaves + primordia)	Number of plants per sample	Mean distance from the apical centre of the youngest primordium ( $\mu\text{m}$ ) <sup>a</sup>	Mean distance from the first primordium below the apical surface ( $\mu\text{m}$ ) <sup>a</sup>	Area of annulus bearing primordia on apical surface ( $10^3 \times \mu\text{m}^2$ ) ( $\pm 1 \text{ s.e.}$ ) <sup>b</sup>	Number of primordia on apical surface	Area of primordium at initiation relative to area of apical surface ( $2\log_e r$ ) <sup>c</sup>	Absolute area of primordium at initiation (apical area $\times 2\log_e r$ ) ( $10^3 \times \mu\text{m}^2$ )
5SD+LD	Reverted	Int. Part/ Leaf (21-29)	6	53.8	134.8	48.9 $\pm$ 5.0	8	0.24	2.2
		Leaf (30-46)	6	65.7	111.3	26.4 $\pm$ 3.1	6	0.18	2.4
		Leaf (47-69)	4	60.5	96.3	20.5 $\pm$ 6.4	5	0.20	2.3
5SD+9LD +SD	Re-flowering	Petal (30-46)	8	64.9	120.9	33.8 $\pm$ 4.6	6	0.23	3.0
		Stamen (47-69)	4	73.3	123.5	31.1 $\pm$ 2.9	6	0.18	3.0

a Geometric means presented; analysis of variance on log transformed data n.s.  
b Reverted Intermediate part/leaf (21-29) plants significantly different from remaining plants at  $P=0.01$ .  
c Analysis of variance on all 5 treatment stages: n.s.



meristem at the Leaf (30-46) and Leaf (47-69) stages (Table 29). The youngest primordium was initiated at about the same radial distance from the apical centre, and hence the bare area of the apical surface remained constant. Similarly, the first primordium below the apical surface was initiated at about the same radial distance from the apical centre in both treatments, and, after the decrease in the area of the annulus bearing the primordia on the apical surface between the Intermediate part/leaf (21-29) and the Leaf (30-46) stages in 5SD + LD, this area remained approximately constant whether petals or stamens were being initiated (5SD + 9LD + SD) or leaves (5SD + LD). After the Intermediate part/leaf (21-29) stage, the number of primordia on the apical surface was about 5-6 in both treatments, and the area of the primordium at initiation (both relative to the area of the apical surface and in absolute terms) was about the same at all stages in both treatments (Table 19).

The conclusion from these data is therefore that, when the reverted plants are returned to SD after 5SD + 9LD, there are no changes in radial growth pattern, as there are when vegetative (LD) plants are transferred to SD on day 0 (see Section 4.2.3, p. 86). The question that then remains is whether this difference in response to SD results from the fact that reverted apices have been altered physiologically from the vegetative (LD) state, because of the 5SD treatment, or whether such a change takes place as a result of ageing of the plants. To answer this question the same measurements were made on transverse



sections of the shoot apex of plants grown in LD until they had initiated about the same number of leaves plus primordia as reverted plants had initiated on return to SD after 5SD + 9LD. In this experiment vegetative (LD) plants were transferred to SD on day 29 and the changes in radial growth pattern over the next 12 days compared with plants remaining vegetative in LD. The results are presented in Table 30.

These results were comparable in most respects to those obtained when vegetative (LD) plants were transferred to SD on day 0 (see Section 4.2.3, Table 15, p. 87 ). As found then, the number of primordia on the apical surface increased on flowering; in this case, from 3 in vegetative plants to 9 by the Mid petal (50-59) stage (Table 30). As before, the area of the primordium at initiation decreased on flowering, so that it was smaller than in the vegetative apex/<sup>both</sup> in relative and in absolute terms, by the Mid petal stage (Table 30). Measurements made on flowering apices at the Bract (35-39) and Early petal (40-49) stages showed that, when transferred at the later stage of vegetative development, the area of the annulus bearing the primordia on the apical surface increased significantly at the time of flowering (Table 30). This increase resulted from an increase in the distance of the first primordium below the apical surface from the apical centre by the Bract (35-39) stage; it was maintained through the subsequent stages of flowering measured here, so that at the Mid petal (50-59) stage the annulus area was significantly larger than in the vegetative apex.



TABLE 30. Area of the apical surface and areas of primordia on initiation in vegetative (LD) and flowering (transfer to SD on day 29) plants sampled between days 29 and 41 after selection for developmental uniformity on day 0. (All data obtained from transverse sections of red-flowered plants of the shoot apex). Data from the Bract/early petal (14-16) stage of plants transferred to SD on day 0 are included for comparison. These data were obtained from S E M photographs and are derived from plants of all flower colours]

Treatment	Type of apex	Stage of development (total no. of leaves + primordia)	Number of plants per sample	Mean distance from the apical centre of the youngest primordium ( $\mu\text{m}$ )	Mean distance from the first primordium below the apical surface ( $\mu\text{m}$ ) <sup>a</sup>	Area of annulus bearing the primordia on the apical surface ( $10^3 \times \mu\text{m}^2$ ) (+ 1 s.e.) <sup>c</sup>	Number of primordia on the apical surface	Area of primordium at initiation relative to the area of the apical surface ( $2\log r$ )	Absolute area of primordium at initiation (apical area $\times 2\log r$ ) ( $10^3 \times \mu\text{m}^2$ )
LD	Vegetative	Leaf	7	63.1	105.0 <sup>b</sup>	22.3 $\pm$ 1.8	3	0.33 <sup>d</sup>	4.1
SD(transferred from LD on day 0)	Flowering	Bract/early petal (14-16)	3	62.7 <sup>f</sup>	120.0 <sup>f</sup>	33.0 $\pm$ 3.3 <sup>f</sup>	3	0.39 <sup>f</sup>	4.8
SD (transferred from LD on day 29)	Flowering	Bract (35-39)	3	61.9	118.6	32.1 $\pm$ 2.4	3	0.42 <sup>d</sup>	5.1
		Early petal (40-49)	5	77.0 <sup>b</sup>	134.2	37.5 $\pm$ 5.0	5	0.23 <sup>e</sup>	4.3
		Mid petal (50-59)	3	68.8	130.2	38.9 $\pm$ 4.7	9	0.15 <sup>e</sup>	2.2

a Geometric means presented; analysis of variance on log transformed data: b is significantly different from remaining treatments at P=0.05. c Analysis of variance - vegetative plants significantly different from remaining plants at P=0.05. d Analysis of variance - significantly different from e at P=0.05. f none of these data is significantly different from the values presented on Table 15, p. 87.



This difference was not found at this stage in plants transferred to SD on day 0 (Table 15, p. 87). Data taken from this experiment for the Bract/early petal (14-16) stage, and included in Table 30 for comparison, show that no statistically significant increase occurred, either in the distance of the first primordium below the apical surface from the apical centre, or in the area of the annulus bearing the primordia on the apical surface, compared with the data from flowering, reverting and vegetative plants presented in Table 15 (p.87 ).

Another difference between flowering on day 0 and flowering on day 29 was that the youngest primordium was initiated significantly further from the apical centre in plants at the Early petal (40-49) stage, not only when compared to vegetative apices, but also when compared with plants at other stages of flowering (Table 30). This was not found in the equivalent stage (Bract/early petal (14-16)) of plants transferred to SD on day 0 (Table 30).

Plants transferred to SD on day 29 therefore showed two differences in radial growth pattern from those transferred on day 0: the annulus area increased from the Bract (35-39) stage onwards, and the area of the apical surface increased transiently at the Early petal (40-49) stage. In plants transferred to SD on day 0 neither of these changes occurred (Tables 15 and 30). However, in other respects flowering in plants transferred to SD on day 29 was similar to that in plants transferred to SD on day 0. In both cases there was a decrease in the area of the primordium at initiation, and an increase in the



number of primordia on the apical surface. These changes were not found when reverted (5SD + 9LD) plants were returned to SD. This indicates that the lack of change in radial growth pattern when reverted apices flowered resulted not from ageing of the plants, but from their previous exposure to SD. The changes in the radial growth pattern that accompanied the transition to flowering of vegetative (LD) plants on transfer to SD on day 0 (Table 15, p.87 ) or day 29 (Table 30) were associated with petal initiation and the attainment of whorled or pseudo-whorled phyllotaxis. Reverted apices appear to have already undergone all the changes in radial growth pattern (Table 29) and phyllotaxis (see Section 5.2.2) characteristic of the transition to flowering, and change no further on re-induction.

#### 5.2.4 Lengths of stem frusta at initiation

The values obtained for frustum length at initiation in the reverted (5SD + LD) apex (Table 31; Fig. 26A-C) of red-flowered plants agreed with those obtained for all flower colours and described previously (see Tables 18 and 19, p.<sup>100</sup>/108). When reverted plants were returned to SD (5SD + 9LD + SD), the length of the frustum at initiation, relative to the length of the tissue generating the frusta, remained statistically indistinguishable from the length in reverted (5SD + LD) plants at equivalent developmental stages (Table 31; Fig. 26A-E). The absolute length of the frustum at initiation was also very similar in both treatments (Table 31). Therefore, a



TABLE 31. Lengths of stem frusta on initiation in reverted (5SD+LD) and re-flowering (5SD+9LD+SD) plants.  
(Data obtained from red-flowered plants only)

Treatment	Type of apex	Stage of development (total no. of leaves + primordia)	Number of plants per sample	Mean distance of the first primordium below the apical surface from the apical surface (length of the tissue generating the frustum)( $\mu\text{m}$ ) (1) <sup>a</sup>	Length of the frustum at initiation relative to the length of the tissue generating the frustum( $\log_e v$ ) ( $\pm 1$ s.e.) (2) <sup>b</sup>	Absolute length of the frustum at initiation (1) x (2) ( $\mu\text{m}$ )
5SD+LD	Reverted	Intermediate part/leaf (21-29)	4	6.8	0.27 $\pm$ 0.03	1.8
		Leaf (30-46)	6	10.1	0.17 $\pm$ 0.02	1.7
		Leaf (47-69)	4	5.4	0.19 $\pm$ 0.02	1.0
5SD+9LD+SD	Re-Flowering	Petal (30-46)	5	7.5	0.17 $\pm$ 0.02	1.3
		Stamen (47-69)	3	12.6	0.16 $\pm$ 0.01	2.0

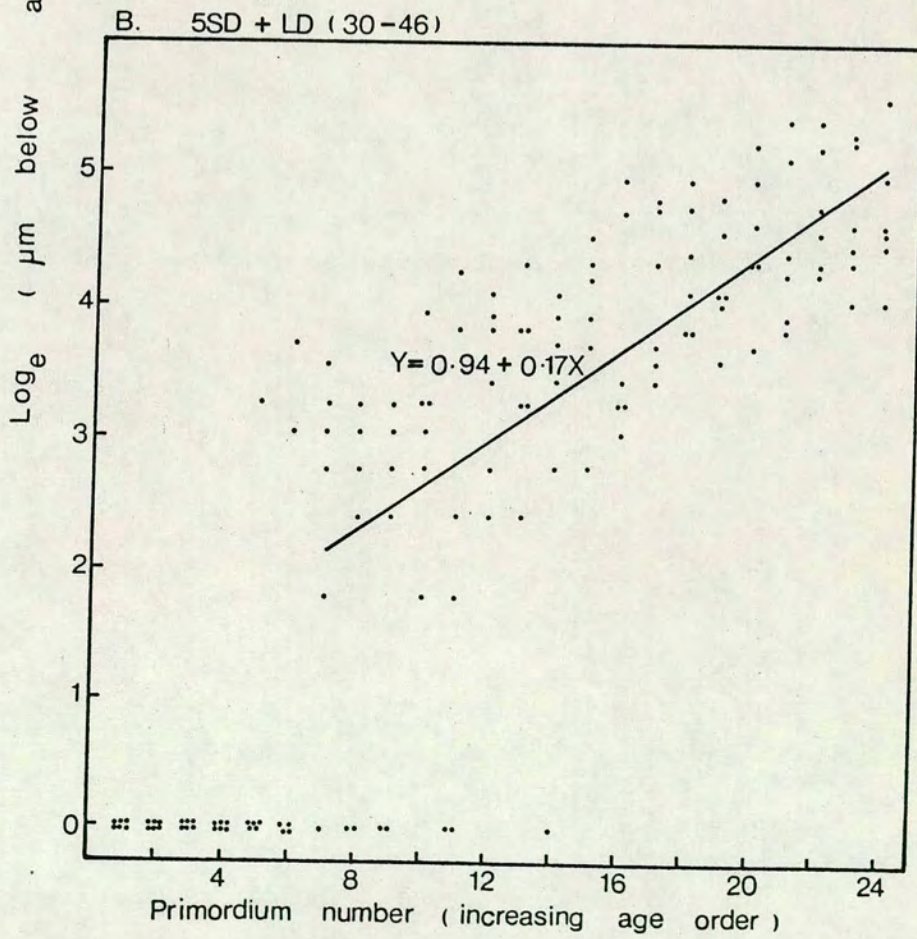
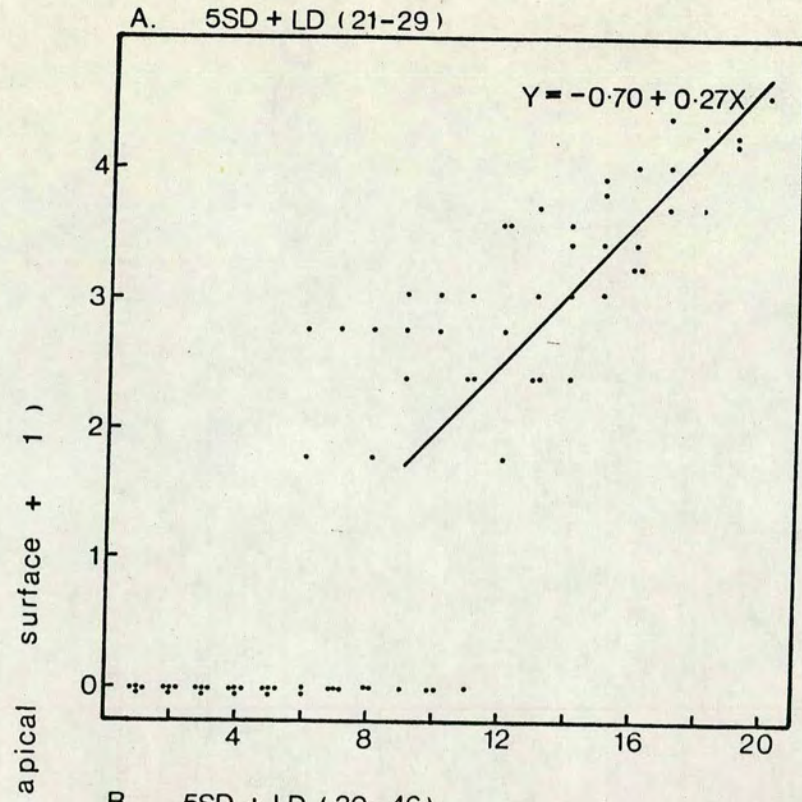
a Geometric means presented; analysis of variance on log transformed data n.s.

b Reverted, Intermediate part/leaf (21-29) plants significantly different from remaining plants at P=0.05.

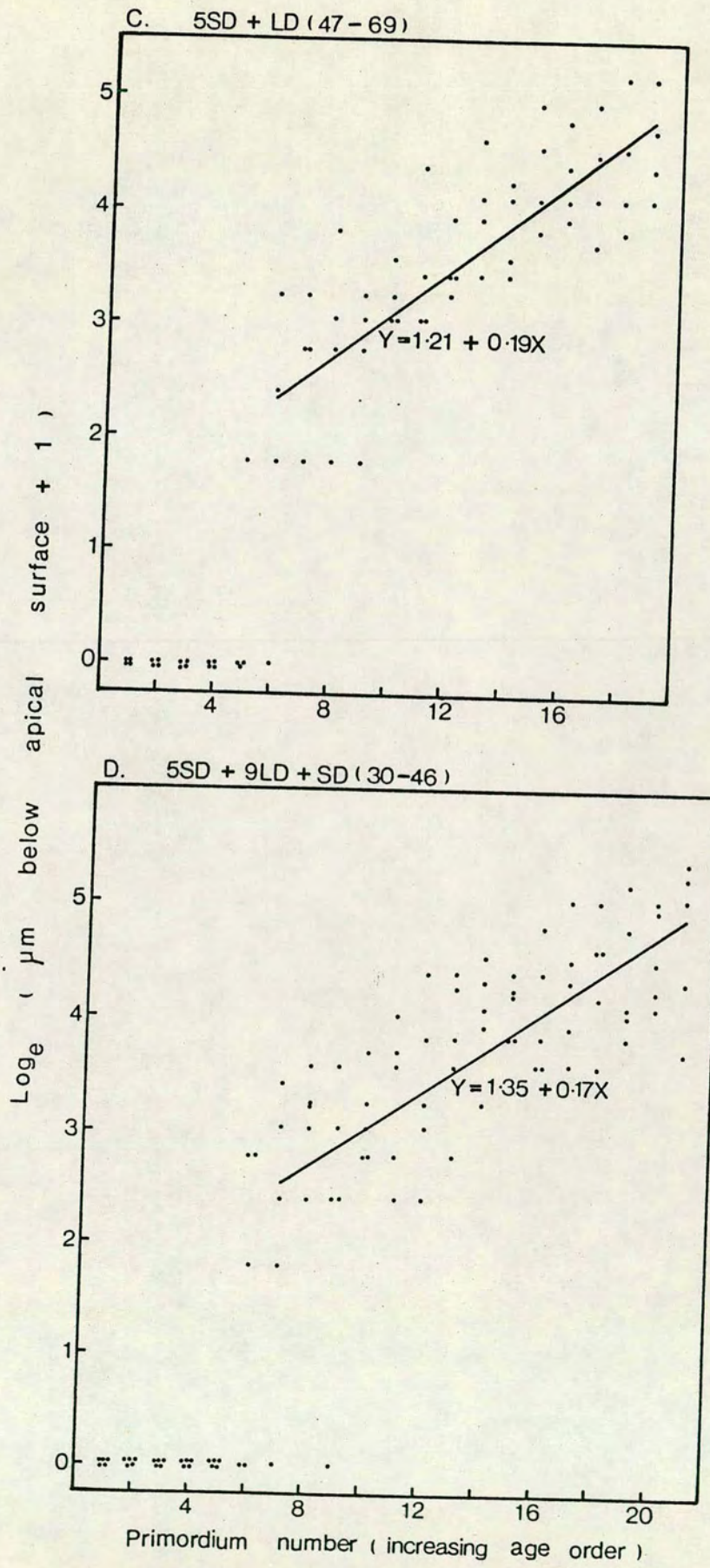


Figure 26. Estimation of the length of the frustum on initiation in reverted (5SD+LD) plants at the (A) Intermediate part/leaf (21-29) stage, (B) Leaf (30-46) stage, (C) Leaf (47-69) stage, and in re-flowering (5SD+9LD+SD) plants at the (D) Petal (30-46) stage and (E) Stamen (47-69) stage.  $\text{Log}_e(\mu\text{m below the apical surface} + 1)$  of the insertion points of primordia from red-flowered plants are plotted as a function of primordium number in each case. For further details of method see legend to Figure 17.

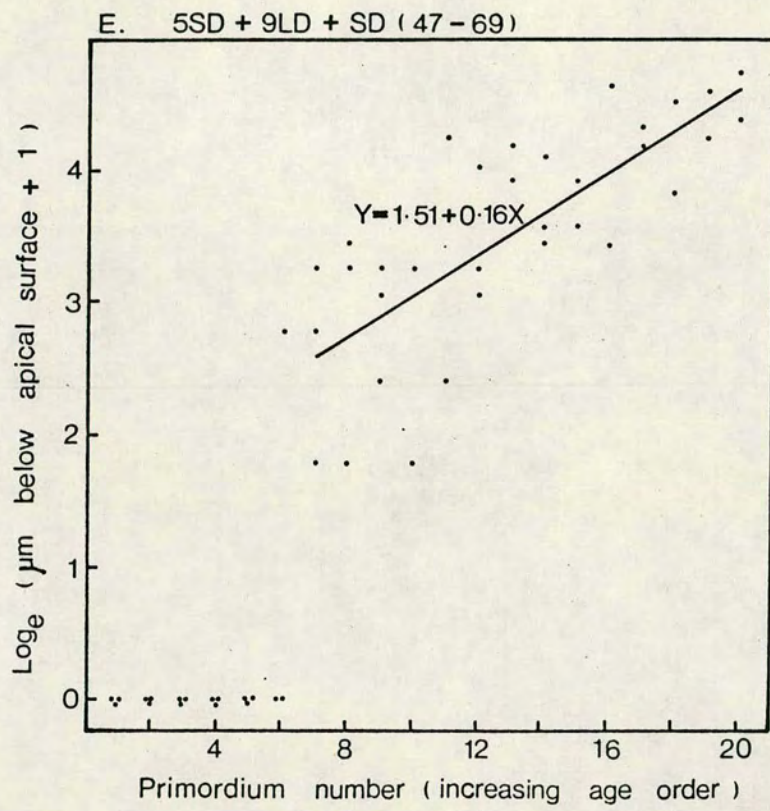














change in frustum length at initiation did not accompany flowering of the reverted (5SD + 9LD) apex, as it did flowering of the vegetative (LD) apex on day 0 (see Section 4.2.4A). To see whether this difference was due to ageing of the plants, frustum length at initiation was estimated in the control experiment, in which vegetative (LD) plants were transferred to SD on day 29. Values could not be obtained for the Bract (35-39) or Early petal (40-49) stages of the flowering (SD) apex because insufficient floral primordia had been initiated to allow regression lines to be fitted to the data. Furthermore, because the rate of primordium initiation in SD was slightly slower than in previous experiments in which plants were transferred to SD on day 0 (Section 5.2.1), no flowering (SD) plants had reached the Late petal (60-64) stage by the last day of sampling. This meant that comparison of frustum length at initiation was limited to vegetative (LD) plants and flowering (SD) plants at the Mid petal (40-49) stage (Table 32; Fig. 27A and B). By the Mid petal (40-49) stage frustum length at initiation was not significantly different from that in vegetative plants (Table 32). This agrees with the results of the previous experiment, when plants transferred to SD on day 0 showed a significant reduction in frustum length at initiation only by the Late petal (30-38) stage (Table 18). However, because of the absence of data for this later stage in plants transferred to SD on day 29, it is not known whether, on flowering in SD after day 29, frustum length at initiation decreased significantly. Such a decrease would,



TABLE 32. Lengths of stem frusta on initiation in vegetative (LD) and flowering (transfer to SD on day 29) plants sampled between days 29 and 41 after selection for developmental uniformity on day 0.

(Data obtained from red-flowered plants only)

Treatment	Type of apex	Stage of development (total number of leaves + primordia)	Number of plants per sample	Mean distance of the first primordium below the apical surface from the apical surface (length of the tissue generating the frustum) (μm) (1) <sup>a</sup>	Length of the frustum at initiation relative to the length of the tissue generating the frustum (log v) $(\pm 1 \text{ s.e.}) e^{-\frac{1}{2}}$ <sup>b</sup>	Absolute length of the frustum at initiation (1) x (2) (μm)
LD	Vegetative	Leaf	10	8.5	0.30 $\pm$ 0.02	2.6
SD	Flowering	Bract (35-39)	3	11.5	-	-
		Early Petal (40-49)	5	5.1	-	-
		Mid petal (50-59)	3	9.2	0.31 $\pm$ 0.05	2.9

<sup>a</sup> Geometric means presented; analysis of variance on log transformed data n.s.

<sup>b</sup> Comparison of regression lines: n.s.



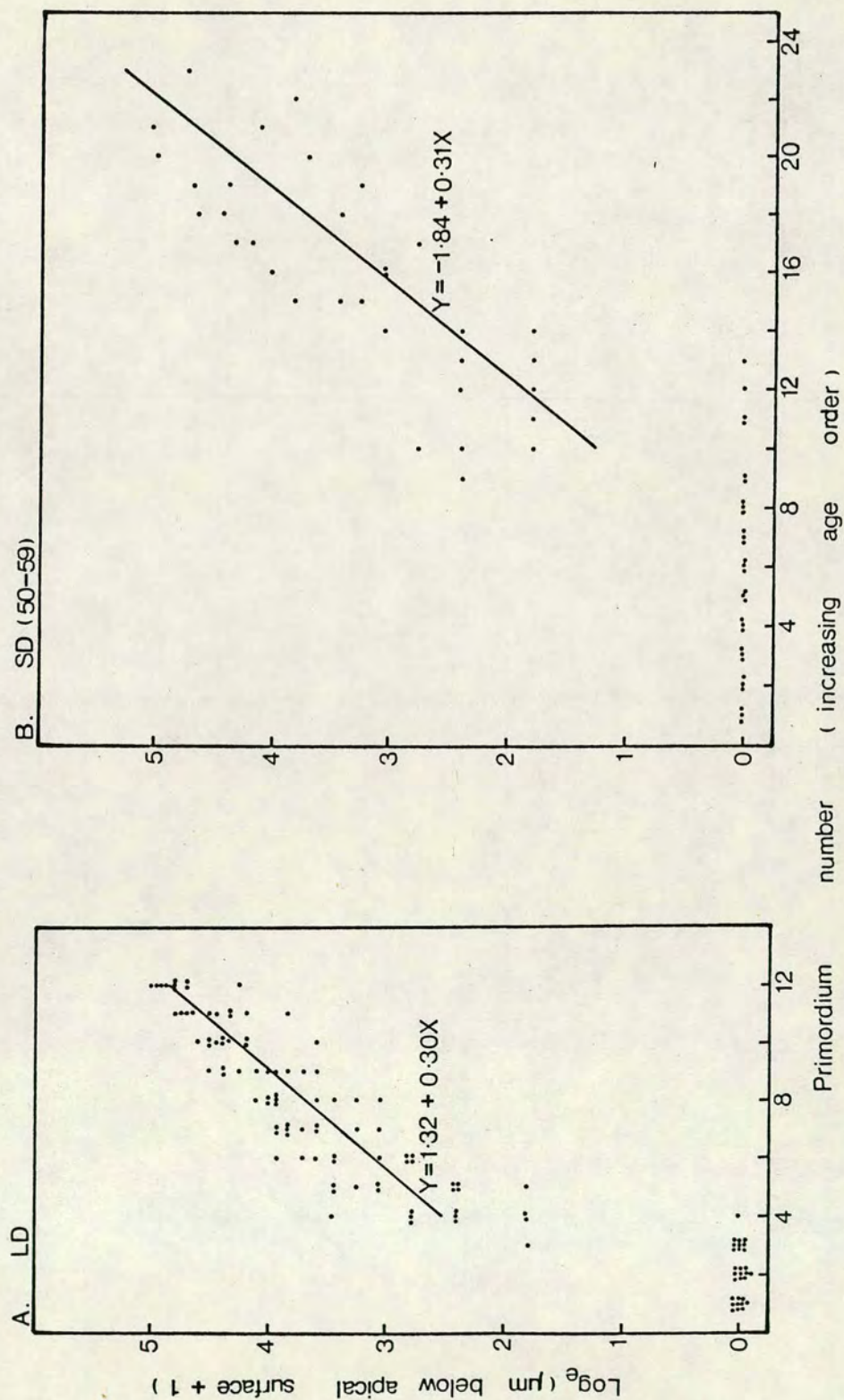


Figure 27. Estimation of the length of the frustum on initiation in vegetative (LD) plants (A) and flowering (SD) plants at the Mid petal (50-59) stage (B). Plants to be induced to flower were transferred to SD on day 29. Log (μm below the apical surface + 1) of the insertion points of primordia from red-flowered plants are plotted as a function of primordium number in each case. For further details of method see legend to Figure 17.



however, be expected, because the changes in radial growth pattern were similar on the transition to flowering on day 0 and day 29 (Section 5.2.3), and the changes in vertical growth pattern were similar at those stages for which data are available (compare Tables 18 and 32).

It can be concluded from these data that, when transferred to SD, the reverted (5SD + 9LD) apex initiated petals and then stamens without any accompanying change in the length of the frustum at initiation (Table 31). This indicates that reduction in the length of the frustum at initiation was not necessarily linked to the initiation of these organs in SD.

A related question is whether frustum length at initiation dictates frustum length (the length of the node + internode below) at maturity. In Section 4.2.4 it was found that, although internodes re-appeared after reversion to leaf initiation, frustum length at initiation remained the same as during reversion, when internodes were absent (Tables 18 and 19, p. <sup>100</sup> 108 & ). This suggested that frustum length at initiation did not determine frustum length at maturity. A similar conclusion is suggested by the mature form of flowers resulting from the 5SD + 9LD + SD treatment, as the following considerations show.

One internode  $\geq$  1 mm long was visible within the mature flower of just over half the plants given 5SD + 9LD + SD (Table 33; Internode 1). The presence of this internode paralleled the return of internodes after reversion in the 5SD + LD treatment (Table 33). However, there was no change in frustum length at initiation in apices in



TABLE 33. Position and lengths of internodes  $\geq$  1 mm long in mature reverted (5SD+LD) and re-flowered (5SD+9LD+SD) plants (Normal flowering (SD) plants have no internodes  $\geq$  1 mm long within the flower). Combined data from four experiments; data obtained from red-flowered plants only

A Number of part at base of internode (mean  $\pm$  1 s.e.)  
B Number of plants with this internode/total number of plants sampled  
C Length of internode (mm) (mean  $\pm$  1 s.e.)

Internodes $\geq$ 1 mm long with <sup>in</sup> the flower (5SD+9LD+SD) or above the reversion zone (5SD+LD)												
Treatment	Internode 1			Internode 2			Internode 3			Internode 4		
	A <sup>a</sup>	B	C	A <sup>a</sup>	B	C	A <sup>a</sup>	B	C	A <sup>a</sup>	B	C
5SD+9LD+SD (Re-flowered) (Dissected day 42-9)	31.1 +1.6 _	8/13	2.6 +0.6 _	37.3 +1.7 _	6/13	1.4 +0.3 _	43.0	1/13	4.0	-	0/13	-
5SD+LD (Reverted, R4) (Dissected day 42-9)	25.7 +0.8 _	13/13	6.5 +1.5 _	27.9 +1.2 _	11/13	6.1 +1.8 _	29.7 +1.4 _	7/13	9.8 +2.6 _	35.0 +3.0 _	5/13	6.0 +1.6 _
5SD+LD (Reverted, R4) (Dissected day 70)	25.1 +1.8 _	7/7	17.0 +7.1 _	28.3 +2.5 _	7/7	28.7 +4.5 _	34.0 +3.2 _	7/7	30.4 +4.9 _	39.7 +4.1 _	7/7	18.4 +2.9 _

a Part 1 = the lowest leaf on the plants



5SD + LD or 5SD + 9LD + SD, correlated with this return of internodes (Table 31).

Furthermore, internodes were rarely found above part 43 in the mature flowers of plants given 5SD + 9LD + SD (Table 33; Internode 3). If this disappearance of internodes resulted from a decrease in frustum length at initiation, then this decrease would be expected in plants sampled at the Stamen (47-69) stage in the 5SD + 9LD + SD treatment, since by this stage frustum length at initiation was estimated from parts 41-63. No such decrease was found, however, either compared with reverted (5SD + LD) plants, or with re-flowered (5SD + 9LD + SD) plants at the Petal (30-46) stage (Table 31).

This constancy of frustum length at initiation during the different stages of development of the reverted and re-flowered meristems shows that frustum length at maturity is not a function of frustum length at initiation. The implication is that frustum length at maturity is controlled at least partly by factors operating after frustum initiation.

#### 5.2.5 Area and vertical relative growth rates per day of the apex

The area relative growth rate of the reverted (5SD + LD) apex was about the same at all three stages measured here (Table 34) as had been found in earlier experiments using plants of all flower colours (see Tables 21 and 22, p. <sup>118</sup> 114 ). There may have been a slight decrease from the Intermediate part/leaf (21-29) stage to the Leaf



TABLE 34. Area and vertical relative growth rates per day of the apical surface and frusta of reverted (5SD+LD) and re-flowering (5SD+9LD+SD) plants. (Data obtained from red-flowered plants only)

Treatment	Type of apex	Stage of development (total number of leaves + primordia)	Area relative growth rate per day ( $2\log_r$ x rate of primordium initiation) <sup>a</sup>	Vertical relative growth rate per day ( $\log_v$ x rate of primordium initiation) <sup>a</sup>
5SD+LD	Reverted	Intermediate part/leaf (21-29)	0.58	0.65
		Leaf (30-46)	0.43	0.41
		Leaf (47-69)	0.48	0.46
5SD+9LD+SD	Re-flowering	Petal (30-46)	0.48	0.36
		Stamen (47-69)	0.38	0.34

<sup>a</sup> Since both area and vertical relative growth rates per day are derived from values (see Tables 29 and 31) which have error terms, standard errors cannot be calculated for the values presented.



(30-46) stage (Table 34), but it seems more probable that this represented variation around a mean value of about 0.5, the same value as found in the earlier experiments (Tables 21 and 22). The vertical relative growth rate per day decreased between the Intermediate part/leaf (21-29) stage and the Leaf (30-46) stage (Table 34), as had been found previously (Table 21), although in this experiment it was more pronounced. On the other hand, the further decrease to a value almost the same as that in vegetative (LD) apices, reported earlier (Table 22), was not evident here (Table 34); the vertical relative growth rate per day was about the same at the Leaf (30-46) and the Leaf (47-69) stages.

On re-flowering in 5SD + 9LD + SD there was no marked increase in the area and vertical growth rates per day, compared with reverted (5SD + LD) plants (Table 34). Such an increase was found in plants transferred to SD on day 0 (Tables 21 and 22), and an increase of about 2- to 3-fold for the area relative growth rate per day, or about 4-fold for the vertical relative growth rate per day, was found in plants transferred to SD on day 29 (Table 35).

Therefore, in the reverted (5SD + 9LD) apex, no change in growth rate accompanied the transition to flowering on return to SD. This lack of change was accompanied by a lack of change in phyllotaxis (Section 5.2.2), in the area of the primordium at initiation (Section 5.2.3), and in the length of the stem frustum at initiation (Section 5.2.4). Therefore, the reverted



TABLE 35. Area and vertical relative growth rates per day of the apical surface and frusta of vegetative (LD) and flowering (transfer to SD on day 29) plants sampled between days 29 and 41 after selection for developmental uniformity on day 0. (Data obtained from red-flowered plants only)

Treatment	Type of apex	Stage of development (total number of leaves + primordia)	Area relative growth rate per day ( $2\log_e r \times$ rate of primordium <sup>a</sup> initiation)	Vertical relative growth rate per day ( $\log_e v \times$ rate of primordium initiation) <sup>a</sup>
LD	Vegetative	Leaf	0.20	0.18
SD	Flowering	Bract <sup>b</sup> (35-39)	-	-
		Early petal (40-49)	0.55	- <sup>c</sup>
		Mid petal (50-59)	0.36	0.74

<sup>a</sup> Since both area and vertical relative growth rates per day are derived from values (see Tables 30 and 32) which have error terms, standard errors cannot be calculated for the values presented.

<sup>b</sup> Since the rate of primordium initiation is changing at this stage (see Figure23B), growth rates per day cannot be estimated.

<sup>c</sup> No estimate of  $\log_e v$  is available for this stage (see Section 5.2.4, p. 155 )



apex appears to have achieved a state whereby it is pre-disposed to flower; the only change (of those measured) that does occur on transfer back to SD is that instead of leaves, floral parts are formed. This contrasts sharply with the marked alterations in phyllotaxis and growth pattern that accompany the transition of the vegetative (LD) apex to flowering on transfer to SD, whether this transfer occurs on day 0 (Section 4.2) or day 29 (Section 5.2).



## CHAPTER 6: DEVELOPMENT OF THE PRIMORDIA UNDER DIFFERENT PHOTOPERIODS

The previous chapters have been concerned mainly with the changes in apical growth and phyllotaxis/in response to different photoperiodic treatments. A related problem is posed by the form of the mature parts produced on flowering, reversion and re-flowering of the plants. The existence of intermediate parts on reverted plants (Figs. 8C, 9B, 22B), in which some areas bear petal pigment, and others are leaf-like, indicates that commitment to either petal or leaf development does not take place at the level of the whole organ. Intermediate parts may result from a change in developmental direction that occurs during the growth of petal primordia. Alternatively, intermediate parts may be a distinct category of organ, their form being determined at, or close to, the time of their initiation. In Section 6.1, two attempts to distinguish between these possibilities are reported. The data obtained also provide evidence on the nature of the reversion response taking place in the 5SD + LD treatment.

A second investigation, reported in Section 6.2, made use of the fact that the reverted (5SD + LD) apex initiates primordia that can develop as petals or leaves, according to the photoperiodic treatment (Section 5.1, p.125). It was considered that study of these primordia as they grew and became committed to leaf development would provide a system in which the effect of photoperiod, and hence floral stimuli, could be analysed in individual primordia,



rather than the whole apex. If such a system could be developed, and the changes underlying commitment to leaf development elucidated, then another approach to study of the mode of action, and the nature, of floral stimuli arriving at these primordia would be available.

6.1 The origin of intermediate parts: is their form determined at initiation, or does it result from a switch from petal to leaf development after primordium initiation?

All the data presented in this section were derived from plants of all flower colours.

6.1.1 Comparative analysis of the changes in shape that occur during the growth of leaf, petal, intermediate part, bract, and reverting bract primordia

At maturity leaves and petals differ markedly from one another in both shape and internal structure (Fig. 28A and B). Petals are about half as thick as leaves, and their mesophyll cells do not show the differentiation into palisade and spongy mesophyll that is found in leaves. There are no chloroplasts visible in light microscope sections of petals (Fig. 28B), whereas in both the palisade and spongy mesophyll of the leaf, chloroplasts are abundant (Fig. 28A). Petal pigment is located in the epidermal cells (Fig. 29B); these cells are typically papillate in transverse section (Figs. 28B, 29B) and in surface view (Fig. 30). Leaf epidermal cells do not contain petal pigment (Fig. 29A), and are not papillate (Figs. 28A, 29A).



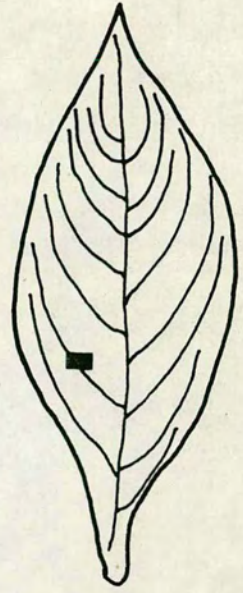
Figure 28. Transverse sections of resin-embedded tissue from (A) a leaf, (B) a petal, and (C) a petaloid area, and (D) a leafy area, of an intermediate part. The areas of the organ from which the sectioned tissue was taken are indicated(■).



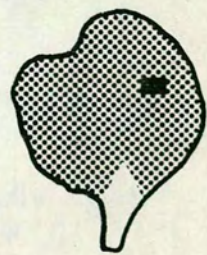
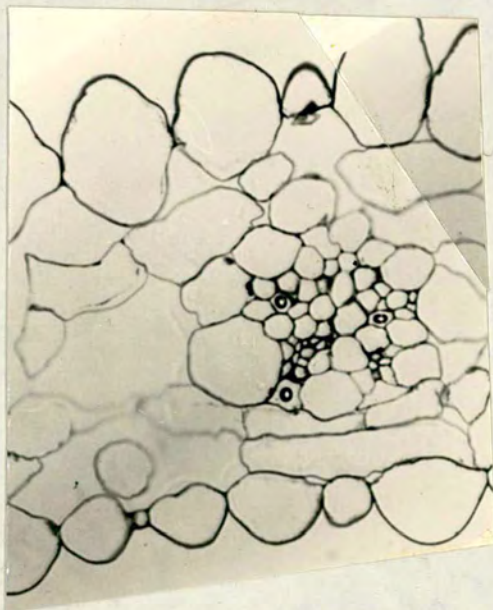
A



100  $\mu\text{m}$

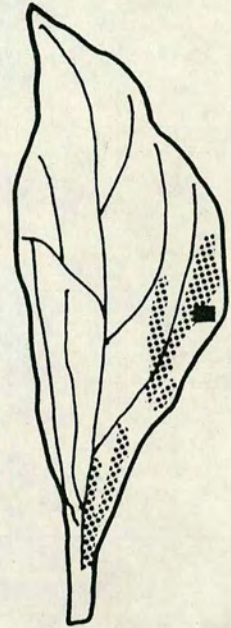
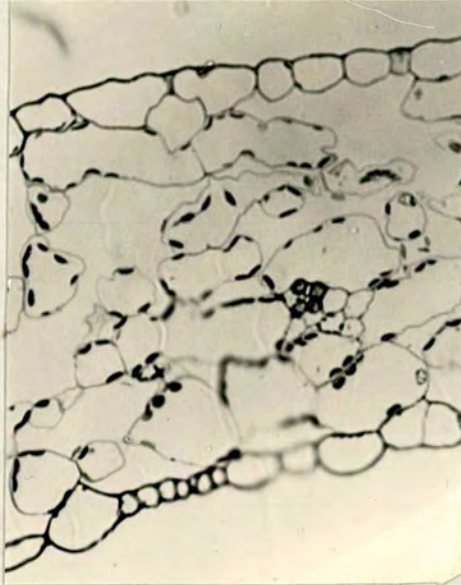


B



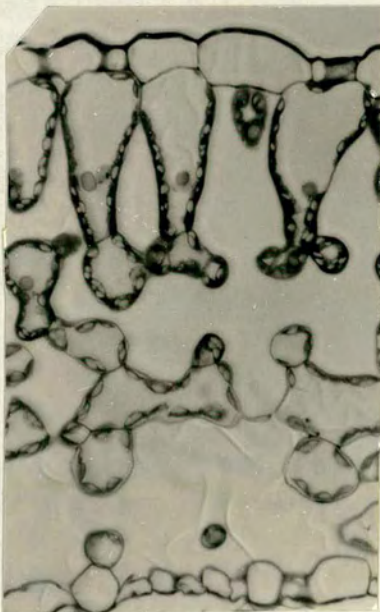


C



100  $\mu\text{m}$

D





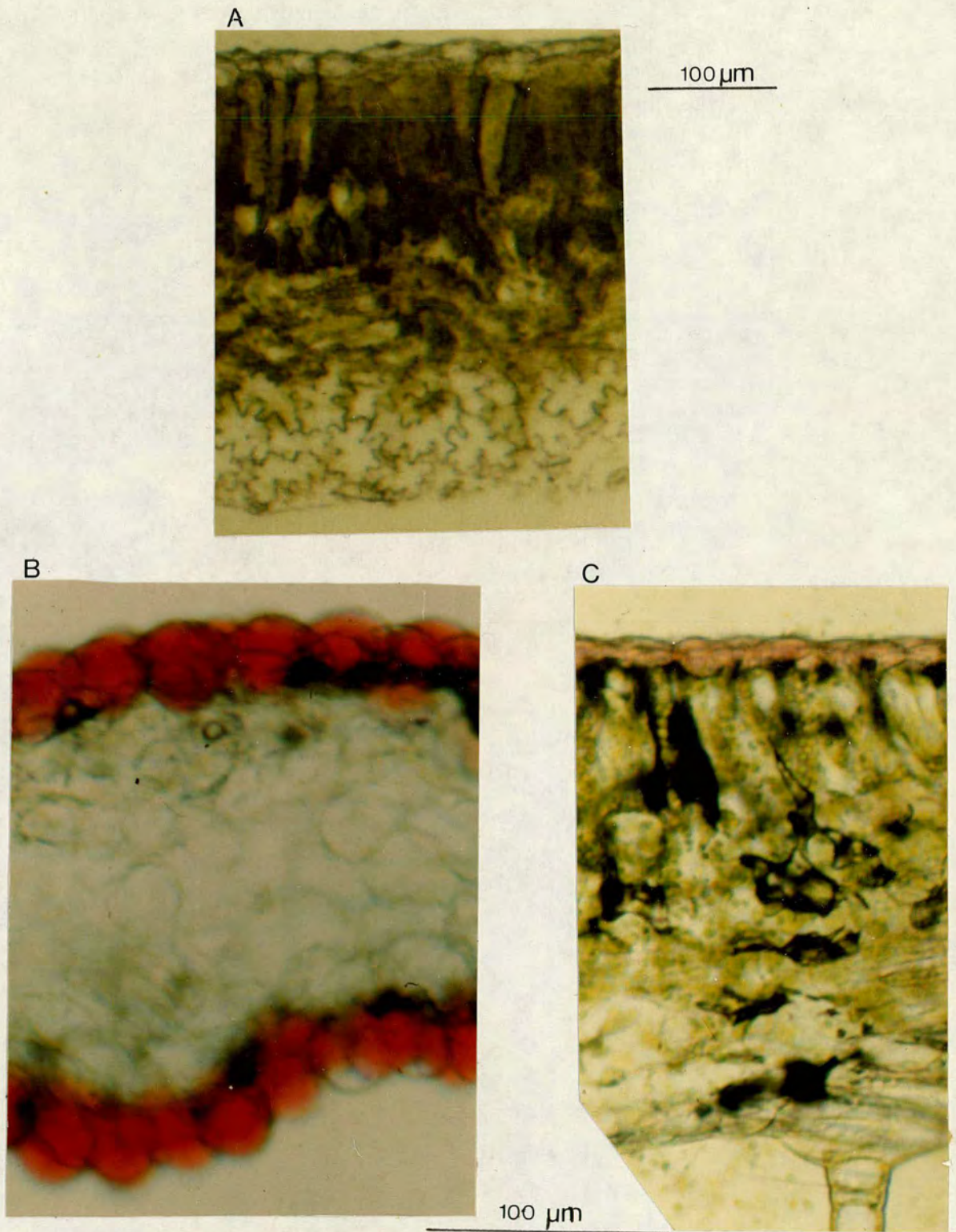


Figure 29. Transverse sections of fresh tissue from (A) a leaf, (B) a petal and (C) a petaloid area of an intermediate part. The tissues were taken from similar areas of the organs to those indicated in Figure 28.



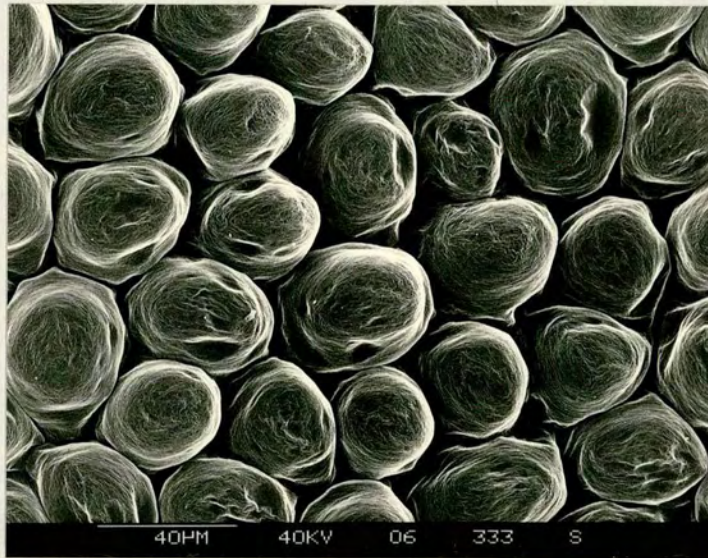


Figure 30. Scanning electron micrograph of the surface of a petal.



Intermediate parts have a shape that is distorted so that it is distinct from both leaves and petals (Fig. 28C). Areas of the epidermis are truly intermediate between the petal and the leaf epidermis: the cells have petal pigment, but their shape is more similar to that of leaf epidermal cells (Figs. 28C, 29C). The internal structure of these areas of intermediate parts shows some differentiation into palisade and spongy mesophyll, and chloroplasts are present (Figs. 28C, 29C). These areas of intermediate parts are about the same thickness as petals (Fig. 28C), whilst areas without petal pigment are typically leaf-like, although/ <sup>not</sup> as thick as leaves (Fig. 28D).

Although these observations pose interesting questions about the control of organ development, it is not possible, from an examination of the mature characteristics of intermediate parts, to determine whether their intermediate nature results from a switch of all but a few remnant areas out of the developmental pathway of petals, during primordium development. The alternative hypothesis, that their heterogeneous form and structure is determined at the time of their initiation, seems equally plausible. In neither case is it clear why certain areas should retain some of the characteristics of petals, whilst the remainder do not.

One approach to the problem of the origin of intermediate parts was to make use of the fact that, at maturity, petals and leaves differ markedly in shape. If a suitable shape index could be developed, so that petal primordia could be distinguished from leaf primordia at



an early stage, then it should be possible to see whether intermediate parts on the reverting apex have an intermediate shape from the time of their initiation. Alternatively, if they begin development as petal primordia, then the stage at which they begin to become more leaf-like could be pinpointed. With this aim in mind, a shape index was developed as follows.

The shape of an organ at maturity is a function of the distribution of growth in its various portions, and of the relative rates of growth of the organ in different dimensions during development (Avery, 1933). It is frequently found that, during development, the ratio of the geometric growth rates of two such dimensions remains constant, even though the ratio of the two dimensions is constantly changing (Simpson, Roe and Lewontin, 1960). When this is the case growth is said to be allometric. If Y and X are the two dimensions measured, the equation of the regression line fitted to the logarithmically transformed data is

$$\log Y = \log a + b \log X,$$

and b is known as the allometric constant. The allometric constant can thus be used as an index of organ shape.

Preliminary measurements of Impatiens leaf and petal primordia during development indicated that approximate allometric relationships existed between length and the width of the primordium halfway between its tip and its base (referred to here as the width), between length and basal width, and between length and primordium area



(Figs. 31, 32, 33). It was easiest to measure length and width, and the distinction between the two organ types was most apparent using these two dimensions. Therefore, it was decided to use length and width to record organ shape. Further measurements of these two dimensions in developing leaf and petal (parts 18-24) primordia indicated that both organs grew allometrically in two distinct phases (Fig. 34), which are in fact apparent in Figure 31. The allometric constant that described each phase (the point of transition from the first to the second phase being estimated subjectively from Fig. 34) in the petal was markedly different from that describing each phase in the leaf (Table 36). The allometric constant therefore provided a very good shape index in this situation.

The same two dimensions were then measured on developing intermediate parts (parts 18-24) from reverting (5SD + LD) plants (Fig. 34), to see at what stage they could first be distinguished from developing petals. The first visible divergence of intermediate parts from petal development had occurred by a length of about 1.0-1.5 mm (Fig. 34); the allometric constant describing intermediate part shape above or at a length of 1.0 mm was intermediate between that for petals  $\geq 1.0$  mm in length and that for leaves  $\geq 1.5$  mm in length (Table 36). Since the exact length at which this divergence of intermediate parts from petal development was estimated subjectively in this method, it was desirable to find a more objective method. One solution was to carry out t-tests on the ratios of  $\log_{10} \text{ width} : \log_{10} \text{ length}$  for a series of length classes



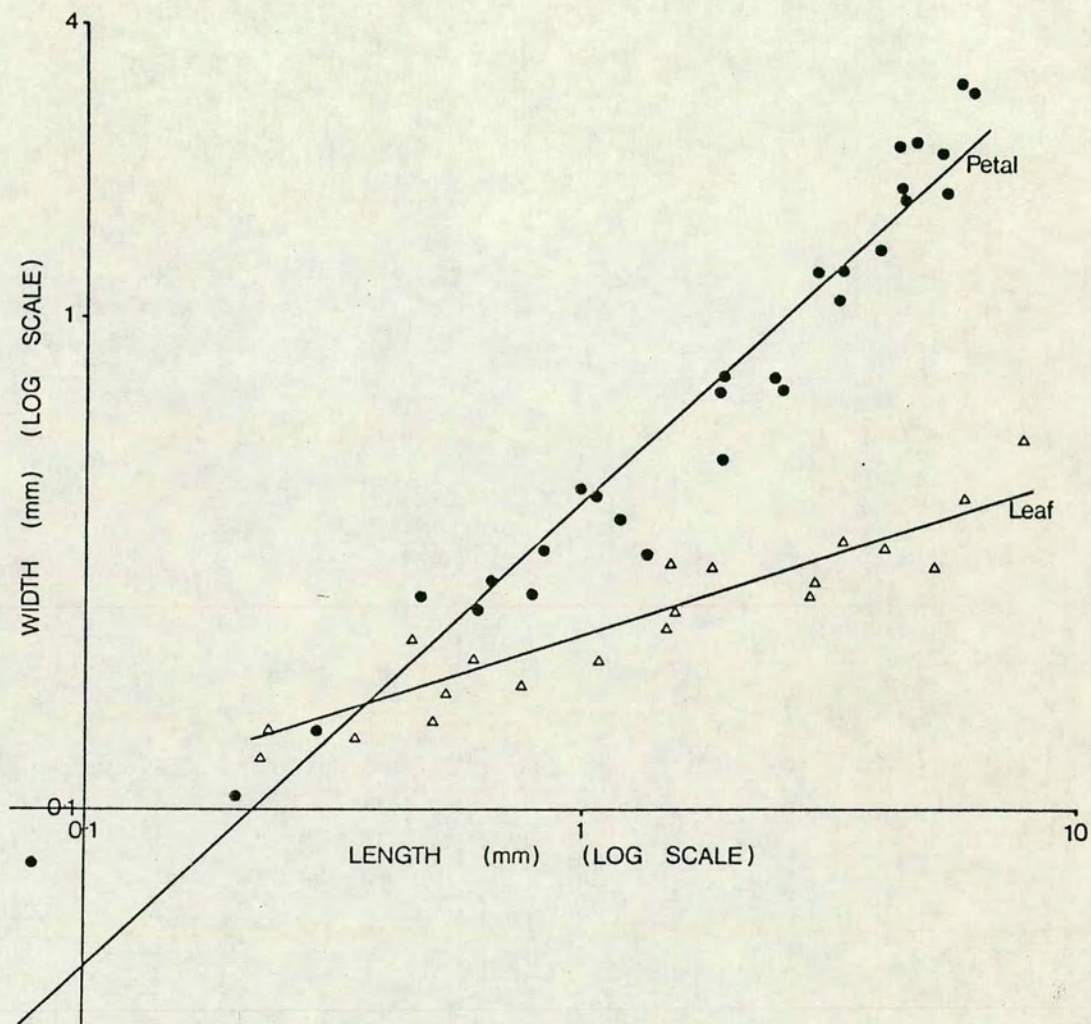


Figure 31. Log-log plot of width against length of leaves from plants growing in LD ( $\Delta$ ) and petals (part 18) from plants growing in SD ( $\bullet$ ).



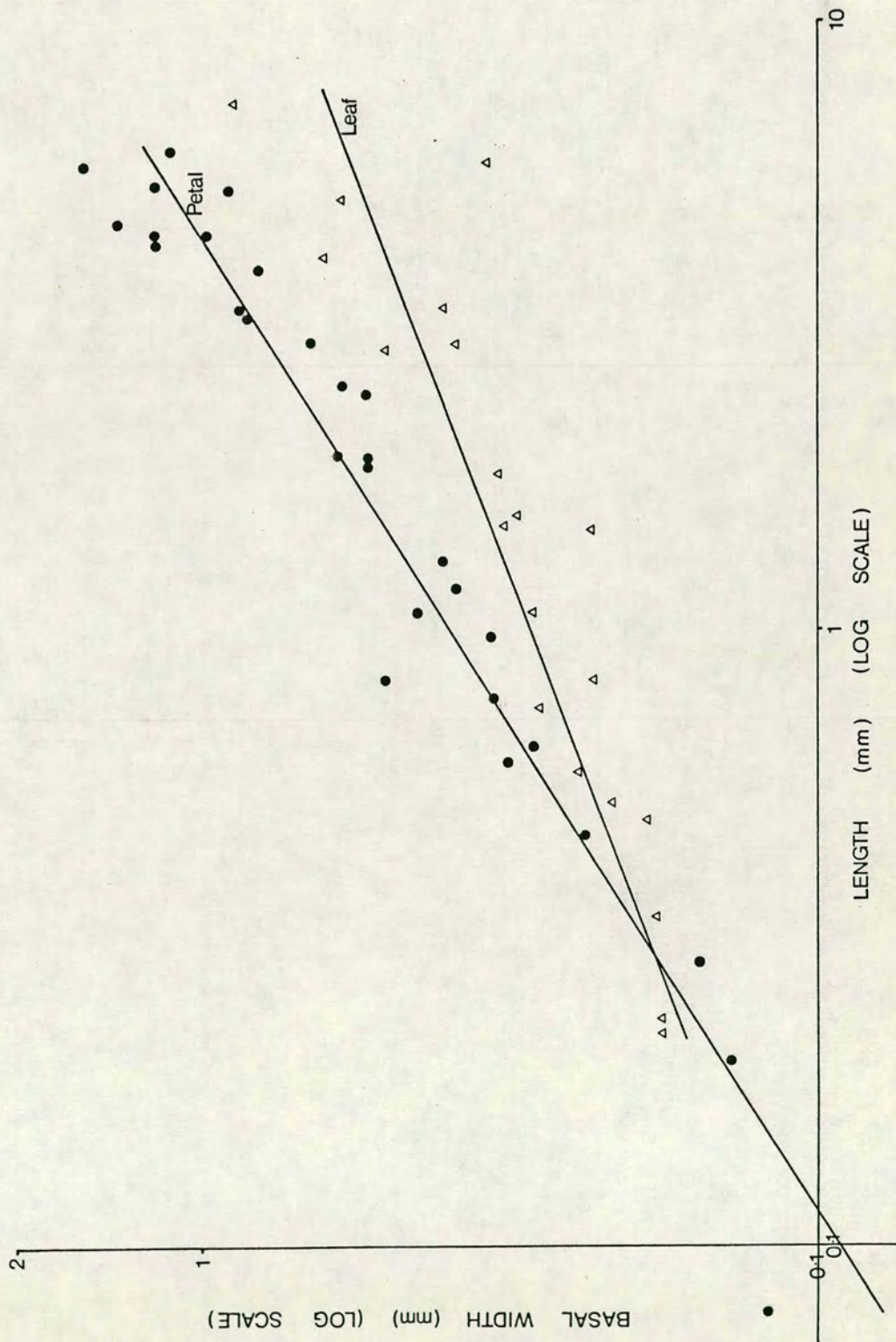


Figure 32. Log-log plot of basal width against length of leaves from plants growing in LD ( $\Delta$ ) and petals (part 18) from plants growing in SD ( $\bullet$ ).



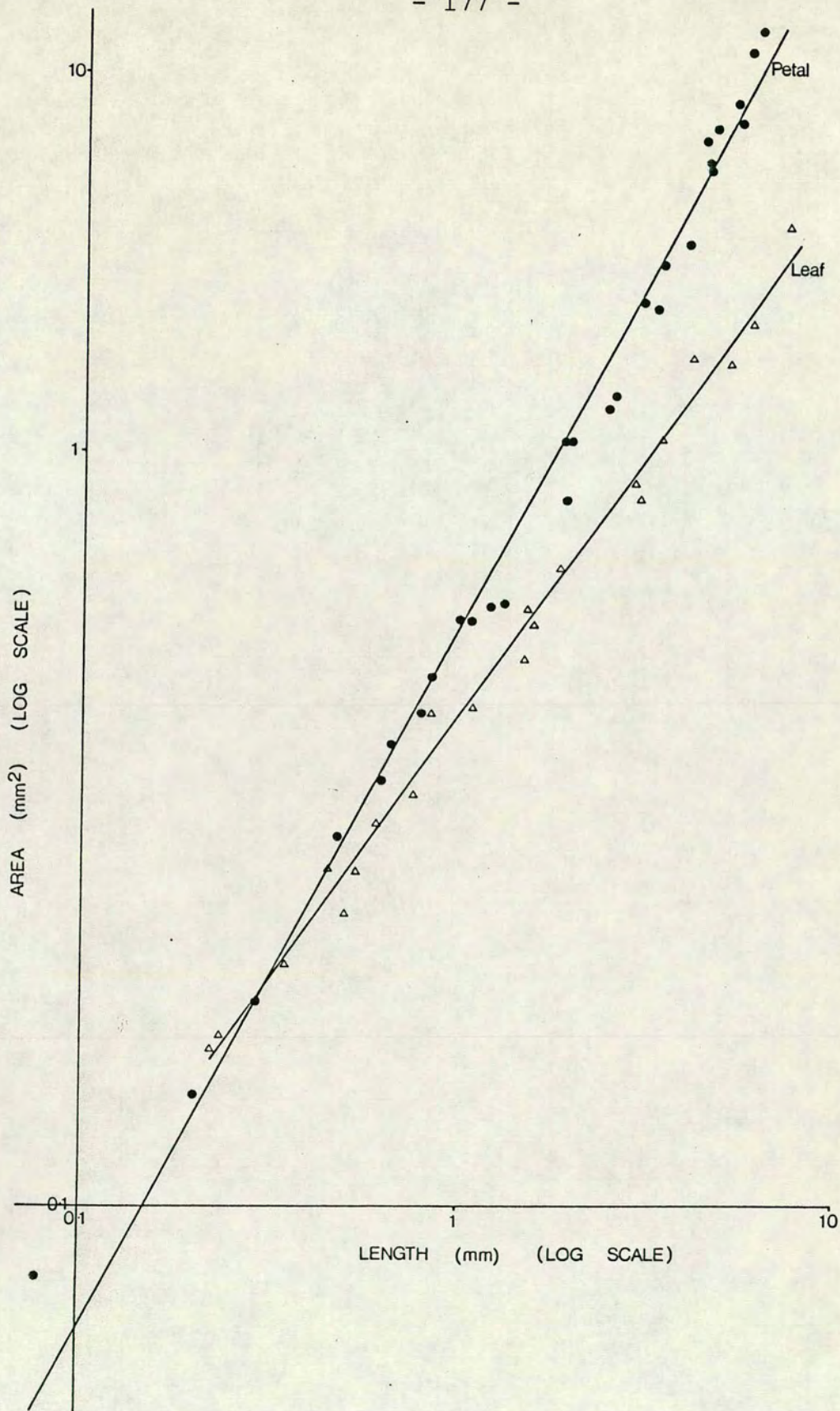


Figure 33. Log-log plot of the area of the upper surface against length of leaves from plants growing in LD (Δ) and petals (part 18) from plants growing in SD (●).



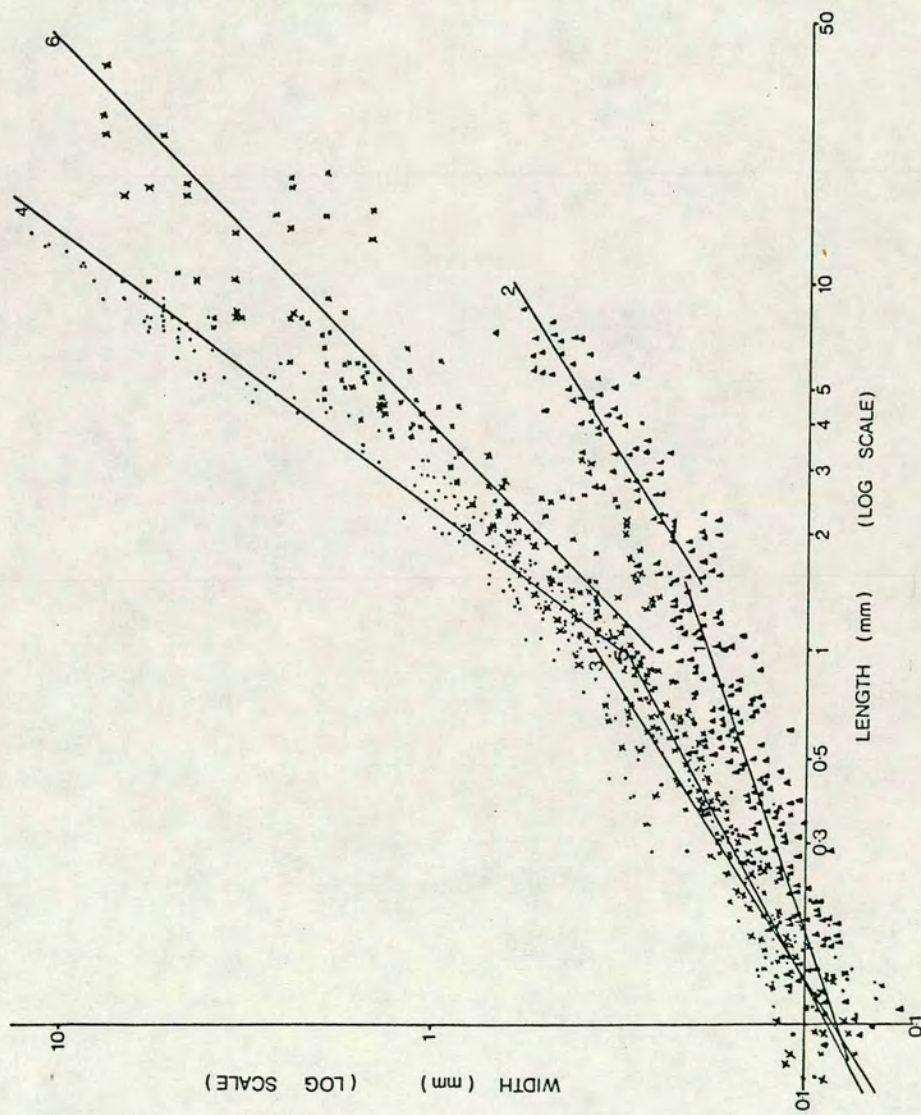


Figure 34. Log-log plot of width against length of leaves from plants growing in LD (▲), petals (parts 18-24) from plants growing in SD (●), and intermediate parts (parts 18-24) sampled, after return to LD, from plants treated with 5SD+LD (x).



TABLE 36. Allometric constants expressing the relationship between  $\log_{10}$  width and  $\log_{10}$  length of parts on vegetative (LD), flowering (SD) and reverting (5SD+LD) plants of all flower colours. The allometric constants are estimated from the regression coefficients from each of the regression lines fitted to the data of Figure 34. The numbers in parentheses (column 4) indicate the regression lines in Figure 34 to which the regression coefficients belong

Treatment	Type of plant part	Length of plant part (mm) below and above which separate regression analyses were performed on each set of data	Regression coefficient (b) <sup>c</sup>	Allometric constant (B) + 95% confidence limits
LD	Leaf	<1.5 ≥1.5	0.35 (1) 0.62 (2)	0.35±0.03 0.63±0.08
SD	Petal (parts 18-24) <sup>a</sup>	<1.0 ≥1.0	0.64 (3) 1.33 (4)	0.64±0.05 1.33±0.04
5SD+LD	Intermediate parts (parts 18-24) <sup>a</sup>	<1.0 ≥1.0	0.54 (5) 0.97 (6)	0.54±0.04 0.97±0.08

<sup>a</sup> Part 1 is the oldest leaf on the plants.

<sup>c</sup> From equations of lines 1 to 6 in Figure 34.

<sup>b</sup> See also Figure 34.

<sup>d</sup> Calculated using the method of Sokal and Rohlf (1969). B may differ slightly from b because it is estimated from the 95% confidence interval.



of intermediate<sup>e</sup> parts and petals. Although it is sometimes inadvisable to perform statistical tests on ratios (Cochran, 1963), it seemed that the method would be less subjective than simple inspection of Fig. 34. It should be stressed, however, that the tests carried out were intended to provide only an indication of the length at which intermediate parts diverged from petal development.

When the t-tests were carried out, it was found that intermediate parts first differed significantly from petals in their  $\log_{10}$  width:  $\log_{10}$  length ratio when between 0.5 and 1.0 mm long (Table 37). Therefore, it can be concluded that intermediate parts had the same shape as petals until they were between 0.5 and 1.0 mm long; at this length they began to change shape, becoming more leaf-like than normal petals.

This conclusion suggests that intermediate parts begin their development as petals; only when they are between 0.5 and 1.0 mm long do they begin to follow a developmental pathway distinct from both petals and leaves. However, this does not rule out the possibility that intermediate parts have their intermediate nature determined at initiation, and that it is the expression of this that begins when they are 0.5-1.0 mm long. If this second possibility were correct, then all reverting primordia would be expected to begin to diverge from their normal pathway of development when between 0.5 and 1.0 mm long. If, on the other hand, intermediate parts begin to diverge from the normal petal developmental pathway when they are between 0.5 and 1.0 mm long because of a



TABLE 37. Mean ratios of  $\log_{10}$  width:  $\log_{10}$  length in different length classes of parts 18 to 24 from flowering (SD) and reverting (5SD+LD) plants. The results of t-tests performed on these data, and the level of significance obtained, are presented

Treatment	Type of plant part	Length (mm) class for which mean $\log_{10}$ width: $\log_{10}$ length ratio was calculated	Mean ratio of $\log_{10}$ width: $\log_{10}$ length	Number of measurements	t	Significance level (P)
SD	Petal	$\geq 0.4 < 0.5$	0.88	16	0.84	n.s.
5SD+LD	Intermediate part		0.87	9		
SD	Petal	$\geq 0.5 < 1.0$	0.88	21	5.02	0.001
5SD+LD	Intermediate part		0.85	61		
SD	Petal	$\geq 1.0 < 1.5$	0.87	45	6.22	0.001
5SD+LD	Intermediate part		0.83	35		



change that occurs over the whole apex at this time then older primordia on the reverting meristem would be expected to diverge from their normal developmental pathway when longer than younger primordia.

These considerations suggested that measurement of the shape of developing bract (SD) and reverting bract (5SD + LD) primordia might provide a way of distinguishing between the two possibilities discussed above. Bracts and reverting bracts (parts 13 and 14) were initiated at least 4 plastochrons before the first petal or intermediate part (part 18) measured in the previous study. This meant that bracts (because they were older) were, during their early development, longer than petals; similarly reverting bracts were longer than intermediate parts (Fig. 35 A and B). Therefore, if it was found that reverting bracts began to diverge from the developmental pathway of normal bracts when 0.5 to 1.0 mm long, the conclusion would be that the changes that take place at this time are probably determined very early on (at initiation). On the other hand, if they began to diverge from the developmental pathway of normal bracts when longer than 0.5 to 1.0 mm, then it would be worthwhile seeing if the time (day after transfer of the plants back to LD) at which they did begin to diverge was the same as that at which intermediate parts began to diverge from petal development.

Measurements of length and width were made on bracts (SD) and reverting bracts (5SD + LD) (parts 13 and 14) during the course of their development. Once again it



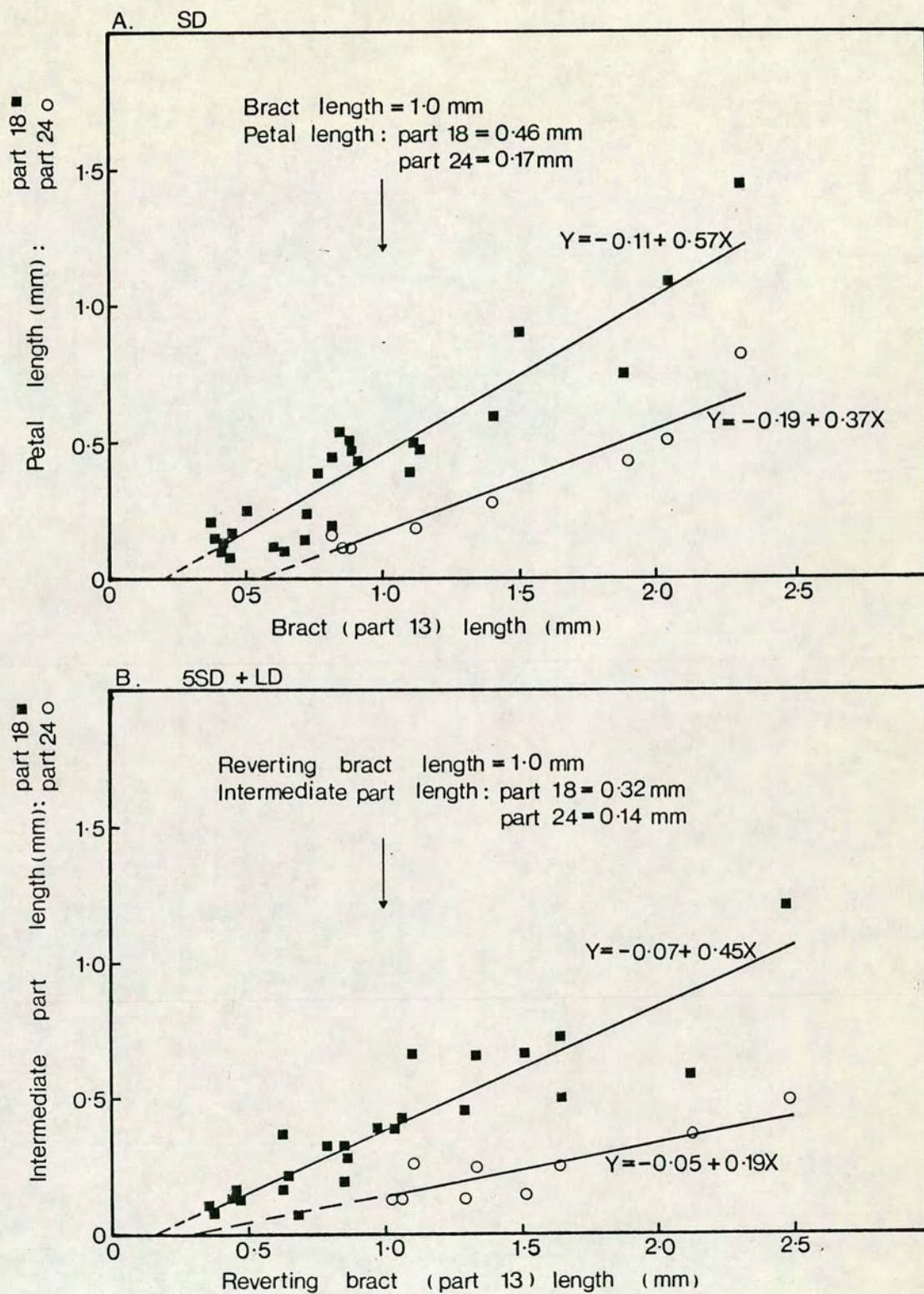


Figure 35. Correlation between the lengths of part 13 and parts 18 (■) and 24 (○) in plants (A) flowering in SD or (B) reverting in LD after a 5SD treatment. Equations for the fitted regression lines are given.



was found that bracts grew allometrically in two distinct phases (Fig. 36), so that the allometric constants provided a good index of bract shape (Table 38). Reverting bracts also appeared to grow allometrically in two phases (Fig. 36); the allometric constant describing their shape was markedly different from that describing normal bract shape during the second phase, when they were  $\geq 1$  mm long (Table 38). To determine when this change in shape first became apparent, t-tests were again carried out on the ratios of  $\log_{10}$  width: $\log_{10}$  length for a series of length classes of bracts and reverting bracts (Table 39). The results indicate that the first significant difference occurred when bracts and reverting bracts were between 1.5 and 3.0 mm long. The relatively small number of replicates in the length class 1.0-1.5 mm meant that the difference at this stage was not significant; inspection of Figure 36 suggests that there may have been some difference at this stage. However, there is no indication, either from Figure 36, or from the t-tests, that bracts and reverted bracts differed in shape when they were 0.5 to 1.0 mm long.

Therefore older reverting primordia (reverting bracts, parts 13 and 14) began to diverge from the developmental pathway of their normal (SD) counterparts when they were longer than younger reverting primordia (intermediate parts, parts 18 to 24). By the previous argument, this suggests that the changes in shape observed are not determined at the time of primordium initiation. The question then remained as to whether reverting bracts began to diverge from their normal counterparts at the same



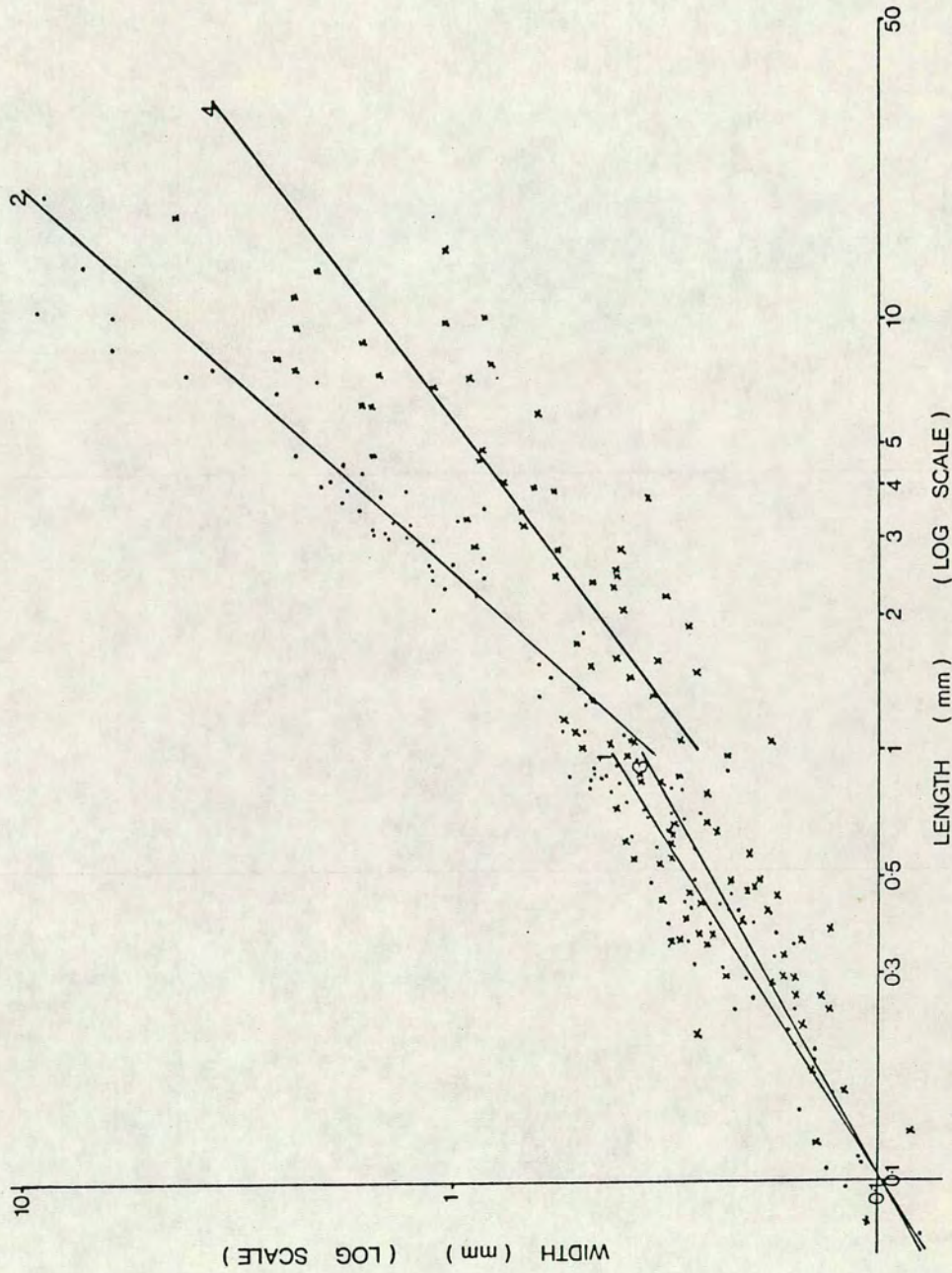


Figure 36. **Log-log** plot of width against length of bracts (parts 13 and 14) from plants growing in SD (●), and reverting bracts (parts 13 and 14) sampled, after return to LD, from plants treated with 5SD+LD (x).



TABLE 38. Allometric constants expressing the relationship between  $\log_{10}$  width and  $\log_{10}$  length of parts 13 and 14 on flowering (SD) and reverting (5SD+LD) plants of all flower colours. The allometric constants are estimated from the regression coefficients from each of the regression lines fitted to the data of Figure 36. The numbers in parentheses (column 4) indicate the regression lines in Figure 36 to which the regression coefficients belong.

Treatment	Type of plant part	Length of plant part (mm) below and above which separate regression analyses were performed on each set of data	Regression coefficient (b)	Allometric constant (B) + 95% confidence limits
SD	Bract (parts 13 & 14) <sup>a</sup>	<1.0 ≥1.0	0.63 <sup>(1)</sup> 1.11 <sup>(2)</sup>	0.63+0.08 1.11+0.11
5SD+LD	Reverting bract (parts 13 & 14) <sup>a</sup>	<1.0 ≥1.0	0.56 <sup>(3)</sup> 0.74 <sup>(4)</sup>	0.56+0.13 0.74+0.13

- <sup>a</sup> Part 1 is the oldest leaf on the plant.  
<sup>b</sup> See also Figure 36.  
<sup>c</sup> From equations of lines 1 to 4 in Figure 36  
<sup>d</sup> Calculated using the method of Sokal and Rohlf (1969). B may differ slightly from b because it is estimated from the 95% confidence interval.



TABLE 39. Mean ratios of  $\log_{10}$  width:  $\log_{10}$  length in different length classes of parts 13 and 14 from flowering (SD) and reverting (5SD+LD) plants. The results of t-tests performed on these data, and the level of significance obtained, are presented

Treatment	Type of plant part (parts 13 & 14)	Length (mm) class for which mean $\log_{10}$ width: $\log_{10}$ length ratio was calculated	Mean ratio of $\log_{10}$ width: $\log_{10}$ length	Number of measurements	t	Significance level (P)
SD	Bract	$\geq 0.5 < 1.0$	0.89	27	1.65	n.s.
5SD+LD	Reverting bract		0.88	18		
SD	Bract	$\geq 1.0 < 1.5$	0.88	6	1.51	n.s.
5SD+LD	Reverting bract		0.84	10		
SD	Bract	$\geq 1.5 < 3.0$	0.87	13	7.37	0.001
5SD+LD	Reverting bract		0.78	15		



time as intermediate parts. This was most easily answered by plotting the lengths of one of the reverting bracts (part 13) against the length of the oldest and youngest intermediate parts measured (parts 18 and 24). When this was done, it was clear that when reverting bracts were between 1.0 and 1.5 mm long (the length at which they may first have begun to differ in shape from normal bracts), intermediate parts (parts 18-24) were between 0.2 and 0.5 mm long (Fig. 35B). Intermediate parts showed the first significant difference in shape from normal petals when they were 0.5 to 1.0 mm long (Table 37). Therefore, reverting bracts and intermediate parts began to differ from their normal counterparts at approximately the same time, with intermediate parts possibly following slightly after reverting bracts. This suggests that the developing primordia on the reverting apex may respond to a change that occurs at a particular time after transfer of the plants back to LD. Reverting bracts were 1.0-1.5 mm long between days 13 and 15 after the beginning of experimental treatments (Fig. 37), about 8-10 days after the transfer of the plants back to LD.

The data are therefore consistent with the idea that a developmental change occurs in the primordia on the reverting (5SD + LD) apex, between days 13 and 15 after the beginning of experimental treatments. The form of intermediate parts probably results from a switch from a petal to a more leaf-like developmental pathway when they are about 0.5 mm long. This agrees with the evidence presented in Section 5.1, p. 125. Here it was shown that



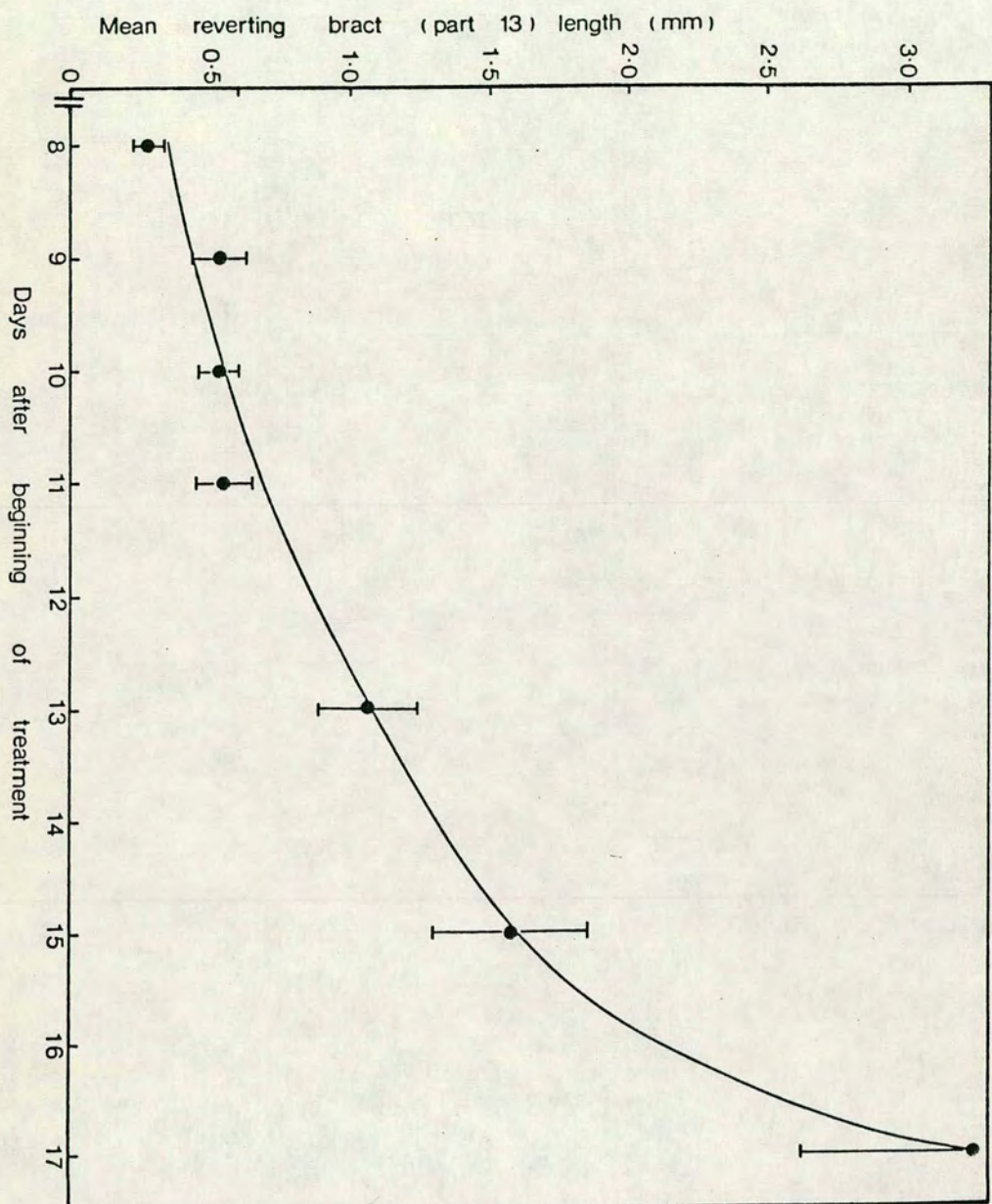


Figure 37. Increase in the length of part 13 (reverting bract) from plants given SSD+LD, between days 8 and 17 following selection for developmental uniformity on day 0. Mean values  $\pm 1$  s.e. are presented.



about 13 primordia present on the reverted (5SD + 9LD) apex at the time of re-transfer to SD were able to switch from the leaf to the petal developmental pathway. The difference in mature form of bracts and reverted bracts (parts 13 and 14) must result from changes operating after their initiation, since they were initiated during the 5SD period, before transfer of the plants to LD (see Fig. 10, p. 75). The evidence presented here suggests that this change may take place when the bracts on the reverted apex are about 1.0 mm long.

#### 6.1.2 Gel electrophoretic study of proteins in leaves, petals and intermediate parts

The second approach to the question of the origin of intermediate parts involved the use of the protein patterns (after one-dimensional gel electrophoresis) of petal and leaf extracts as markers of the two organ types, rather than shape. If petals showed distinctive protein bands on SLS gels of crude extracts made from them, and if these distinctive bands were present early on in petal development, then they could be used as markers of the petal developmental pathway. Extracts of developing intermediate parts could be made, and the time at which they switched from the petal to the leaf developmental pathway (if such a switch occurred) identified using the protein marker bands.

The protein patterns on SLS gels of leaf and petal extracts were found to differ in the relatively mature organs ( $\geq 6$  and  $\leq 80$  mm long). These differences are



shown for organs at different stages during maturation in Figure 38A. The putative large and small sub-units of ribulose biphosphate carboxylase/<sup>-oxygenase</sup> (molecular weights of approx. 50,000 and 15,000 Daltons, respectively) are present in large amounts in leaves; as petals mature in these proteins, always less prominent than/leaf extracts, become progressively less visible (Fig. 38A). This would be expected, given the absence of chloroplasts in mature petals (Fig. 28B). Furthermore, two other protein bands, with molecular weights of approx. 46,000 and 65,000 Daltons, are relatively more prominent in petals than in leaves (Fig. 38A).

The protein complements of petals and leaves therefore differed markedly, even when these organs were fairly small (though macroscopically visible). This suggested that the protein pattern on an SLS gel could be used to distinguish between different organ types. However, when much smaller organ primordia were extracted and the protein extracts run on SLS gels, no consistent differences between leaves, petals and intermediate parts could be detected (Fig. 38B). This might have been because the modified extraction procedure used for organ primordia (see Section 2.8, p.43 ), meant that no differences were apparent between the different organ types. However, several different extraction procedures were used; none of these yielded, in the young primordia, distinctive differences of the sort that were evident between leaves and petals at maturity, and that are shown in Figure 38A. This suggests that, although there may have been differences



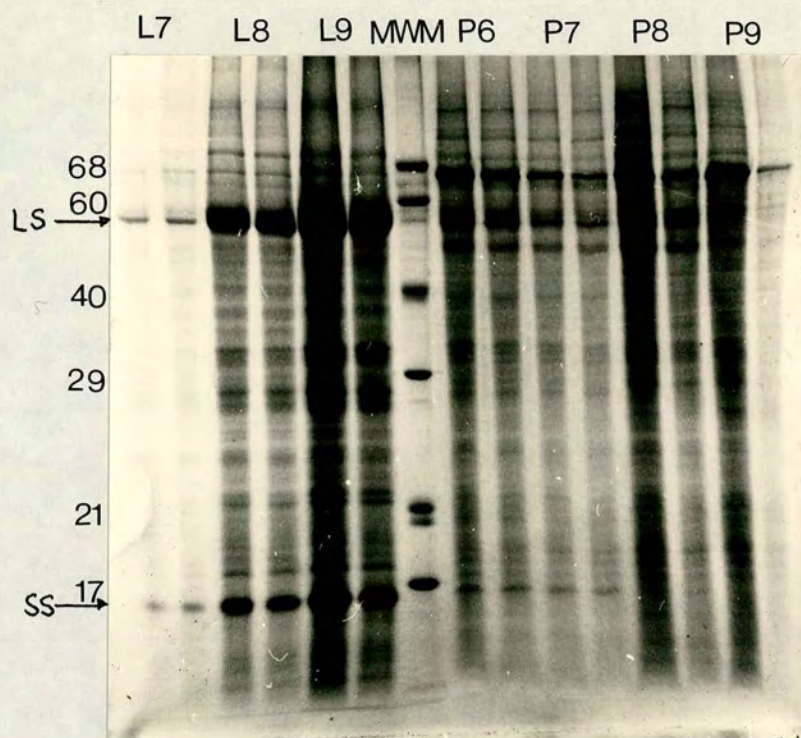
Figure 38. (A) SLS polyacrylamide gel of protein extracts from leaves of plants in LD and petals of plants in SD. Leaf stages (lengths): L7:-1.0-2.0 cm; L8:- 3.0-4.0 cm; L9:- 7.0-8.0 cm; Petal stages (lengths): P6:- 0.6-0.8 cm; P7:- 0.8-1.0 cm; P8:- 1.0-1.6 cm; P9:- 3.0 cm. Two loadings for each stage, one of 50 µg and one of 100 µg of protein per lane. Proteins were stained with Coomassie Blue.

(B) SLS polyacrylamide gel of protein extracts from leaf (LD), petal (SD) and intermediate part (5SD+LD) primordia. Primordium size ranges: 14SD and 5SD+9LD, 150-1000 µm; 20SD and 5SD+15LD, 1500-3000 µm; LD, 500-1500 and 1000-4000 µm. 15 µg total protein loaded in each lane. Proteins were stained with silver nitrate, following the method of Merrill et al (1981) see Section 2.8.2, p.30 ).

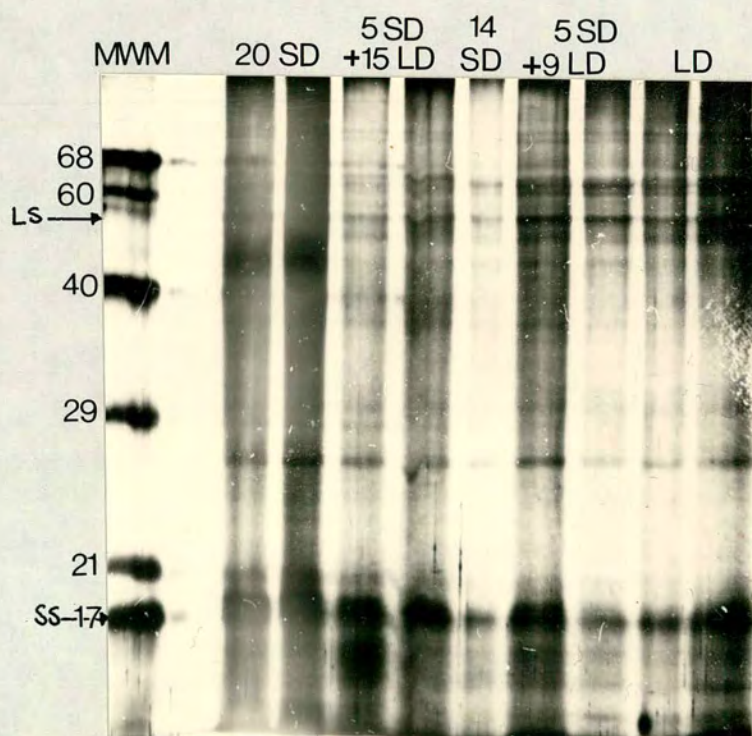
The numbers to the left of each photograph indicate the molecular weights in Kilodaltons of the molecular weight marker proteins (M W M; lane 7 in (A), lane 1 in (B)). The arrows indicate the positions of the putative large (LS) and small (SS) subunits of ribulose biphosphate carboxylase-oxygenase.



A



B





in the protein complement of leaves, petals and intermediate parts during the stages of primordium development studied, the methods employed here were not sufficiently sensitive to detect them.

Therefore the one-dimensional gel electrophoretic protein patterns of crude cell extracts of the different organ types could not be used to distinguish between the organ types early in their development. A more sensitive technique, such as two-dimensional gel electrophoresis, might have revealed subtle differences between petal, leaf and intermediate part primordia.

#### 6.2 The time of commitment of primordia to leaf development on the reverted (5SD + LD) apex. Stereological analysis of cellular changes occurring at the time of commitment

The results presented in this section were obtained from plants with red flowers, because of the uniformity of the reversion response obtained in these plants (see Section 3.3.2, p. 65).

The data presented in Section 6.1.1 indicated that intermediate parts on the reverted apex may exist as a result of a change from petal to leaf development during their early growth. This raised the following general question: at what stage during growth of the primordia initiated by plants given 5SD + LD does irreversible commitment to either petal or leaf development occur?

To answer this question it was most convenient to study the process of commitment in the primordia initiated after reversion had occurred. This was because the potential



leaf primordia initiated by reverted apices had been shown to be uncommitted, so that re-transfer to SD on day 14 caused the youngest 13 primordia present at the time of re-transfer to develop as petals (see Section 5.1, p.125). However, it is clear from inspection of Fig. 21A and B, Section 5.1 (p.126) that, for any given primordium on the reverted apex (e.g. part 33), this totally uncommitted state did not last indefinitely. As the number of LD before re-transfer to SD increased, so the effect of re-transfer on the development of this primordium diminished. The simplest interpretation of this was that as the primordia aged they became committed to leaf development.

Investigation of the time of this commitment was carried out on parts 32, 33 and 34 initiated by the reverted (5SD + LD) apex. These parts were treated as component members of a single whorl or pseudo-whorl; it was assumed that they were identical, and could therefore be treated as within-plant replicates. They were chosen because they were initiated on about day 14 (Fig. 20, p.122), and had been shown to develop as leaves if the plants were left in LD, but to develop as petals if the plants were returned to SD (Section 5.1, p.125).

To study the effect of re-transfer to SD on the development of these primordia as they aged, groups of reverted (5SD + LD) plants were transferred back to SD at intervals between days 14 and 25. The plants were then left in SD until the terminal flower was fully open. The percentage area of the surface of parts 32-34 that was



occupied by petal pigment was then recorded. The results are presented in Figure 39A. As can be seen, there was a decline in the percentage area of the surface occupied by petal pigment in these parts, as transfer back to SD was made at successively later stages in their development. In Figure 39B the mean length of these primordia, obtained from serial transverse sections (Section 2.6.7, p.27 ) and longitudinal sections (Section 2.7.3, p.33 ), is shown.

As the length of the primordia at the time of transfer back to SD increased, the percentage area of their surface occupied by petal pigment at maturity decreased. When transfer back to SD occurred on day 14, the day of initiation of part 33 (Fig. 20, p.122), the primordia developed as normal petals. By day 17 the primordia were about 90  $\mu\text{m}$  long; transfer back to SD at this time resulted in parts with about 63 per cent of their surface occupied by petal pigment at maturity. This indicates that some areas of the primordia became committed to leaf development at an early stage in primordium development. By day 19 the primordia had an estimated length of about 175  $\mu\text{m}$ ; transfer back to SD at this time resulted in parts with about 30 per cent of their surface occupied by petal pigment at maturity. More than half of the primordium had therefore become committed to leaf development by this stage. Commitment of the primordia to leaf development was complete by day 25, when they were, on average, about 750  $\mu\text{m}$  long. The first part of the primordium to become committed to leaf development was usually the tip, and the last parts were around the base. This is illustrated in Figure 40,



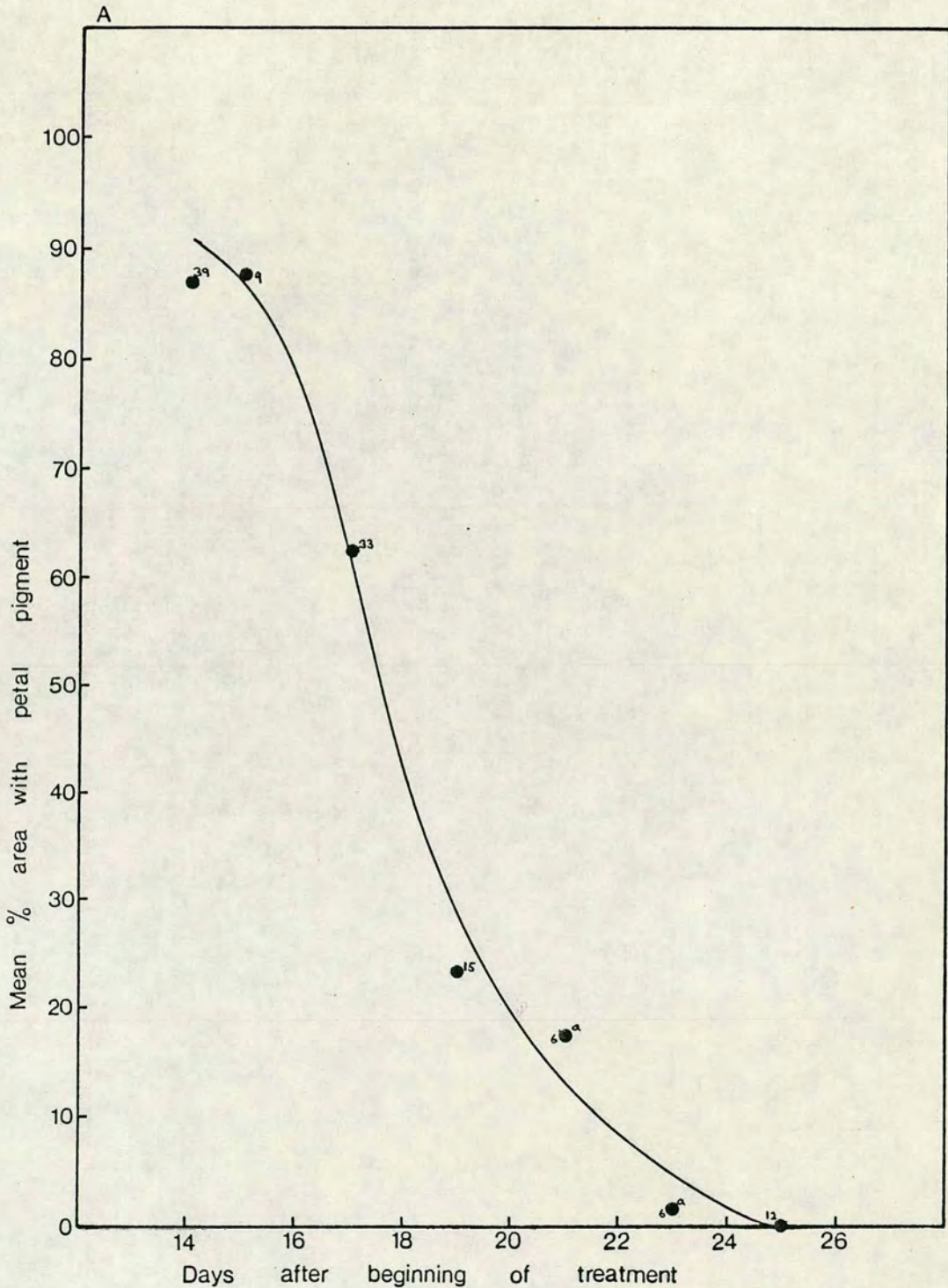


Figure 39(A). Effect of re-transfer of reverted (5SD+LD) plants to SD, after increasing number of LD, on the percentage of the surface area of parts 32-34 occupied by petal pigment at maturity. The number beside each point indicates the number of replicates in each sample. Combined data from five experiments. a: In the 5SD+16LD+SD treatment one plant, and in the 5SD+18LD+SD treatment two plants, showed no evidence of reversion - normal petals were formed from about part 15-17 onwards. These plants were therefore not included in this analysis.



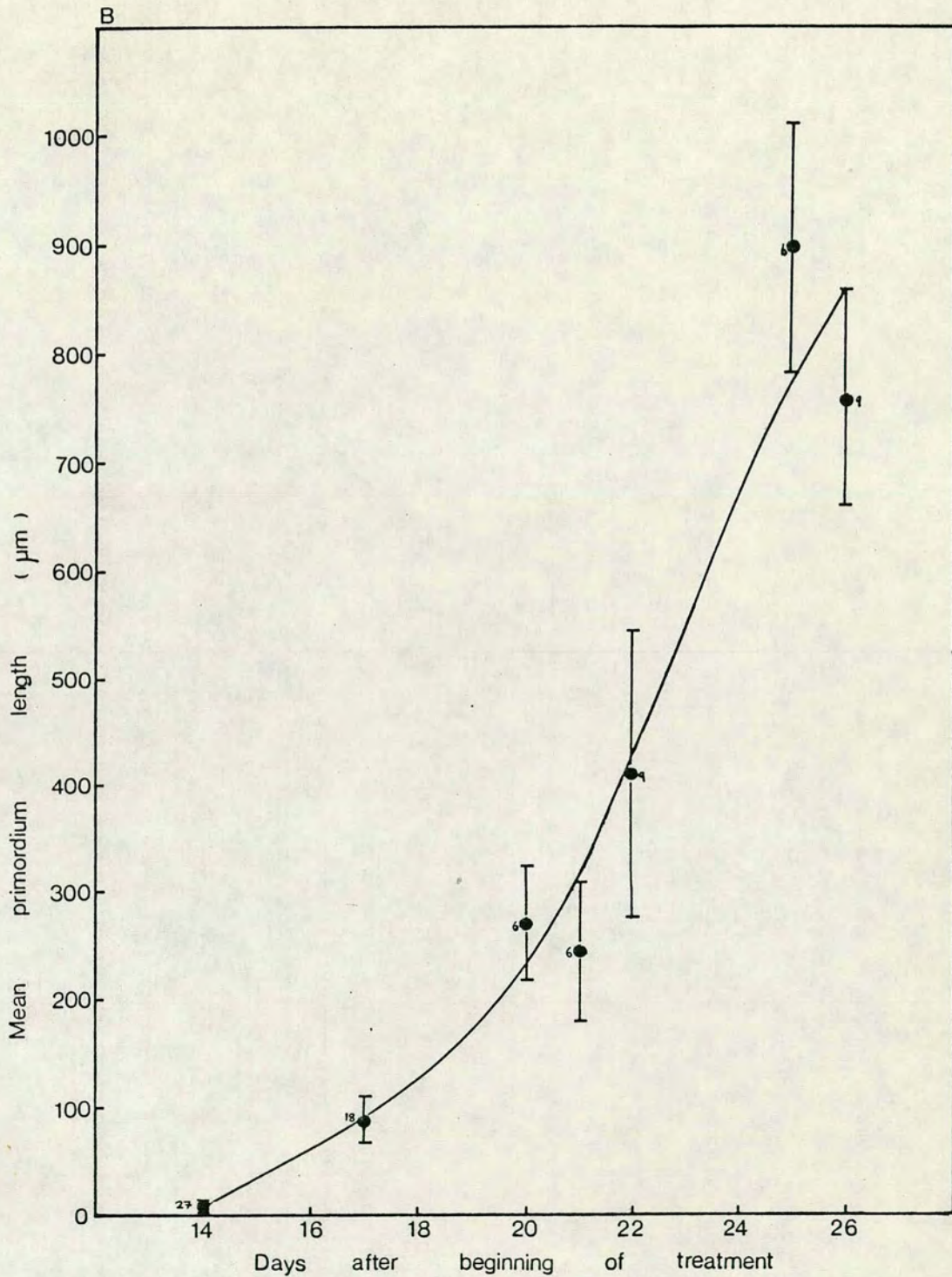


Figure 39(B). Increase in the length of parts 32-34 of reverted (5SD+LD) plants between days 14 and 26 after selection for developmental uniformity on day 0. Mean values  $\pm 1$  s.e. are presented. The number beside each point indicates the number of replicates in each sample. Combined data from three experiments.



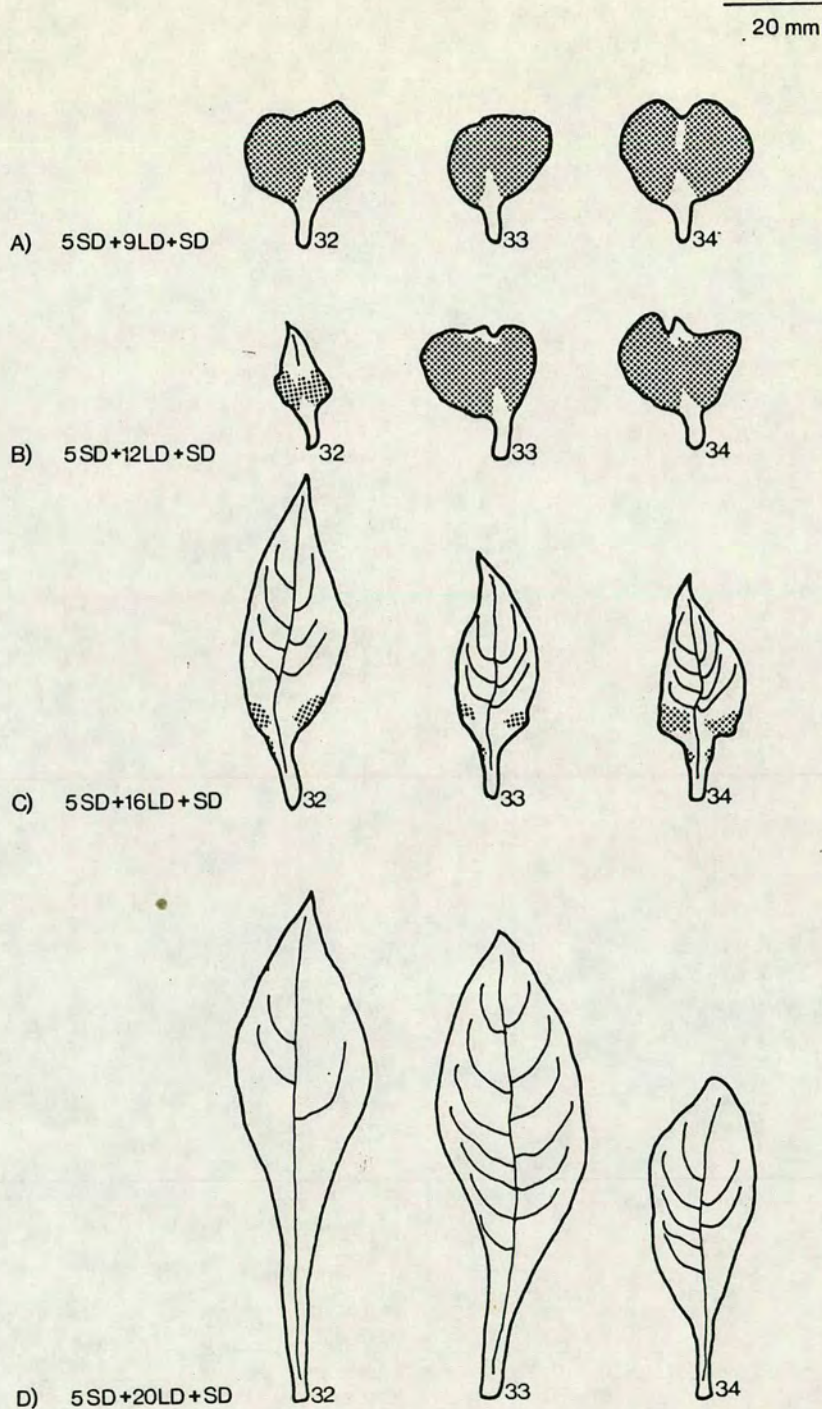


Figure 40. Illustrations of the distribution of petal pigment (shaded areas) in parts 32-34 from reverted (5SD+LD) plants returned to SD after (A) 5SD+9LD, (B) 5SD+12LD; (C) 5SD+16LD; (D) 5SD+20LD. Each part is labelled with its part number. (Part 1 = the lowest leaf on the plants.)



in which examples of the typical pigment distribution at maturity in parts 32-34, from plants given increasing numbers of LD before return to SD, are presented.

It should be stressed that, in this study of the time of commitment of developing primordia, the percentage area of the surface occupied by petal pigment at maturity was used as a marker of the extent of commitment of the primordia to leaf development at the time of re-transfer to SD. Since intermediate parts are known to have characteristics of both leaves and petals (see Fig. 28C and 29C, p. 169 and 170), it is clear that the presence or absence of petal pigment in the epidermal cells represents only one aspect of the effect of photoperiod on the developing primordia. This was used as a marker of commitment in this study because it could be measured most accurately and conveniently.

A further point should be emphasised. The finding that commitment occurred as the primordia increased in length from 0-750  $\mu\text{m}$  long, and was complete when they were more than 750  $\mu\text{m}$  long, depends on the assumption that the effect of re-transfer to SD on the developing primordia is an immediate one. If there is a delay between re-transfer to SD and the effect of SD reaching the primordia, then commitment would occur at a greater primordium length. The data obtained thus give an indication of the earliest time during primordium development that commitment takes place.

When assessed in terms of the percentage of the surface of mature parts occupied by petal pigment, and assuming an immediate effect of re-transfer



to SD, commitment to leaf development took place over a period of about 11 days. During this time the primordia grew from 0 to about 750  $\mu\text{m}$  in length. Furthermore, commitment to leaf development typically occurred basipetally, the tip becoming committed before the base.

These findings prompted the following question: when does commitment to leaf development occur in relation to the changes known to occur in the mesophyll cells during leaf development? These changes typically include vacuolation and differentiation of the cells into palisade and spongy mesophyll cells (Dale, 1982), and an increase in the number of plastids per cell (Possingham and Saurer, 1969; Boffey, Ellis, Sellden and Leech, 1979). If commitment occurs after these changes begin, this would imply that re-transfer to SD accomplishes their reversal, to give the petaloid characteristics found at maturity. If commitment occurs before these changes begin, then it may actually initiate them. In this case the implication is that re-transfer to SD brings about an alteration in the effects of commitment on cell differentiation. If commitment actually coincides with the beginning of vacuolation and cell differentiation, and/or the increase in plastid numbers per cell, then it might be equivalent to them; this would be consistent with the observation that some cells in intermediate parts have characteristics of both leaf and petal cells (Fig. 28C, 29C, p.169 and 170). This question was considered important, not only in terms of understanding commitment itself, but also because, if the nature of <sup>the</sup>/commitment step could be established, then the



nature of the changes which render the cells of the primordium incapable of responding to SD (floral) stimuli would be known. This in turn might point to the nature of the stimuli themselves.

The changes in cell ultrastructure occurring during the time the primordia (parts 32-34) became committed to leaf development were therefore measured by stereological techniques. Four areas were selected for analysis, two at the tip and two at the base of the primordia, to detect any differences that might coincide with the basipetal pattern of primordium commitment. These areas were located 1/4 (areas a and b) and 3/4 (areas c and d) of the way down the primordia; analysis was confined to the two layers of cells immediately below the adaxial (areas a and c) and abaxial (areas b and d) epidermides (Fig. 41; see also Section 2.7.3, p. 33).

To allow measurement of changes in nuclear, cytoplasmic and vacuolar volume, as well as changes in plastid numbers and volumes, the analysis was carried out at two levels. In the level 1 analysis the percentage of the cell occupied by cytoplasm, vacuole and nucleus was measured on low power transmission electron micrographs. The results for each of the four areas are presented in Fig. 42A-D; the change in appearance of the cells of each area during primordium development is illustrated in Fig. 43 A-C. In all four areas the percentage of the cell occupied by vacuole increased, and the percentage occupied by cytoplasm decreased, as primordium length increased. These changes were most marked after the primordia were



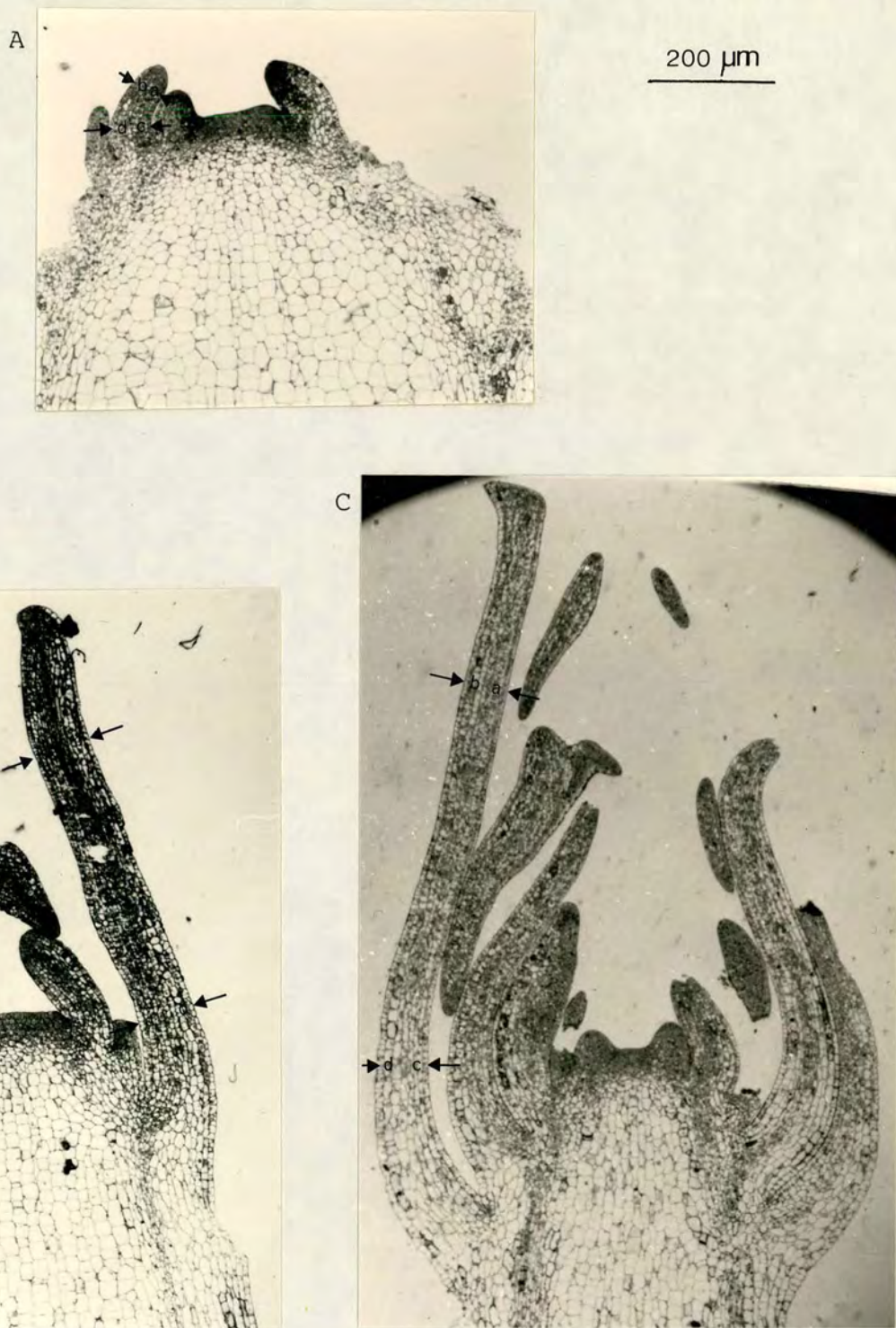
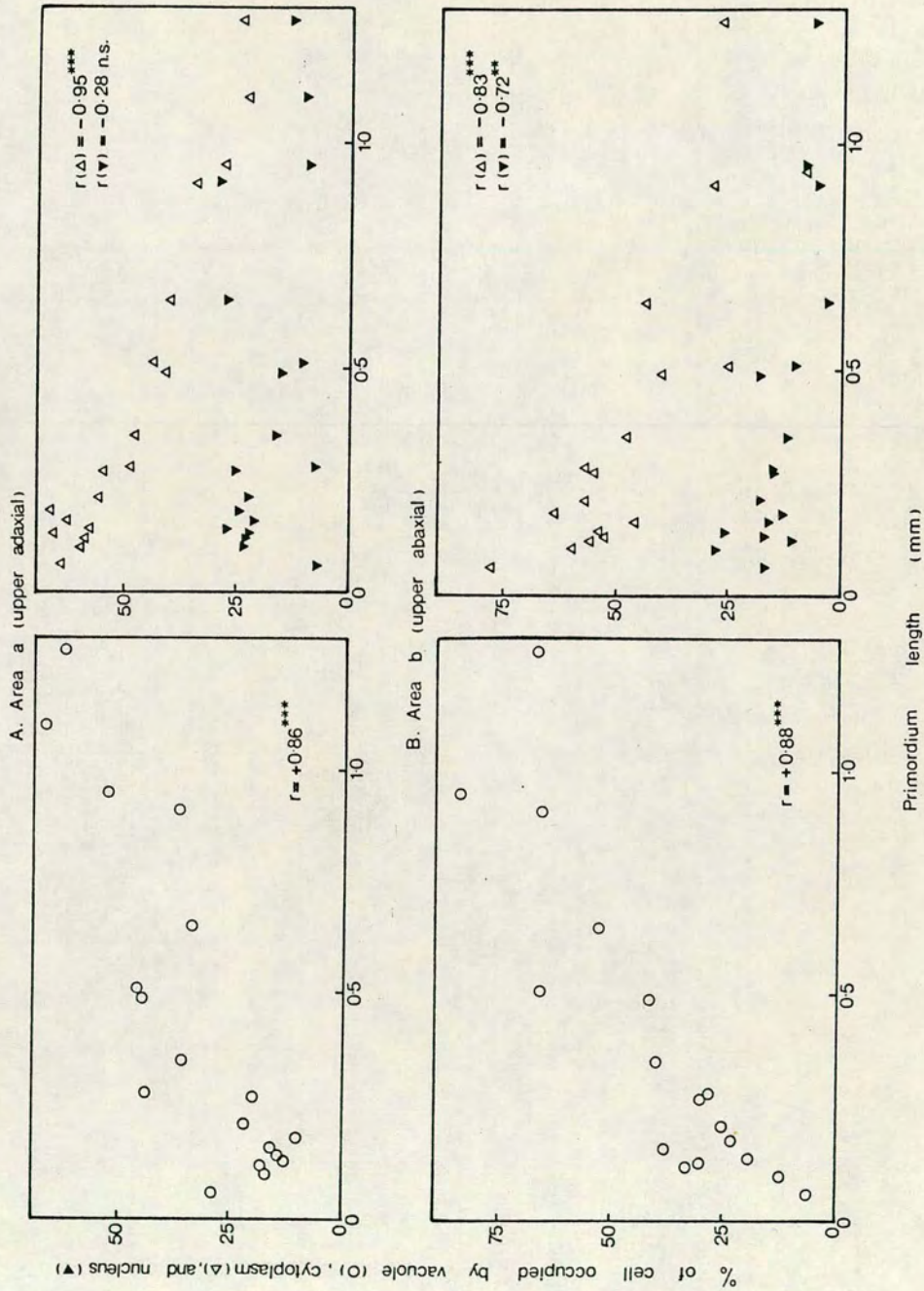


Figure 41. Examples of the location of areas a-d in longitudinal sections of parts 32-34, from reverted (5SD+LD) plants. The primordia are 0.21 (A), 0.91 (B), and 1.27 (C) mm long.



Figure 42. Changes in the percentage of cell volume occupied by vacuole (o), cytoplasm ( $\Delta$ ), and nucleus ( $\blacktriangledown$ ), with increasing primordium length, in cells from areas a (A), b (B), c (C) and d (D). The correlation coefficients (r), with the level of significance, are given for each parameter in each area. \*\*\*, \*\*, \*, significant at  $P=0.001$ , 0.01 and 0.05, respectively. n.s. - not significant.







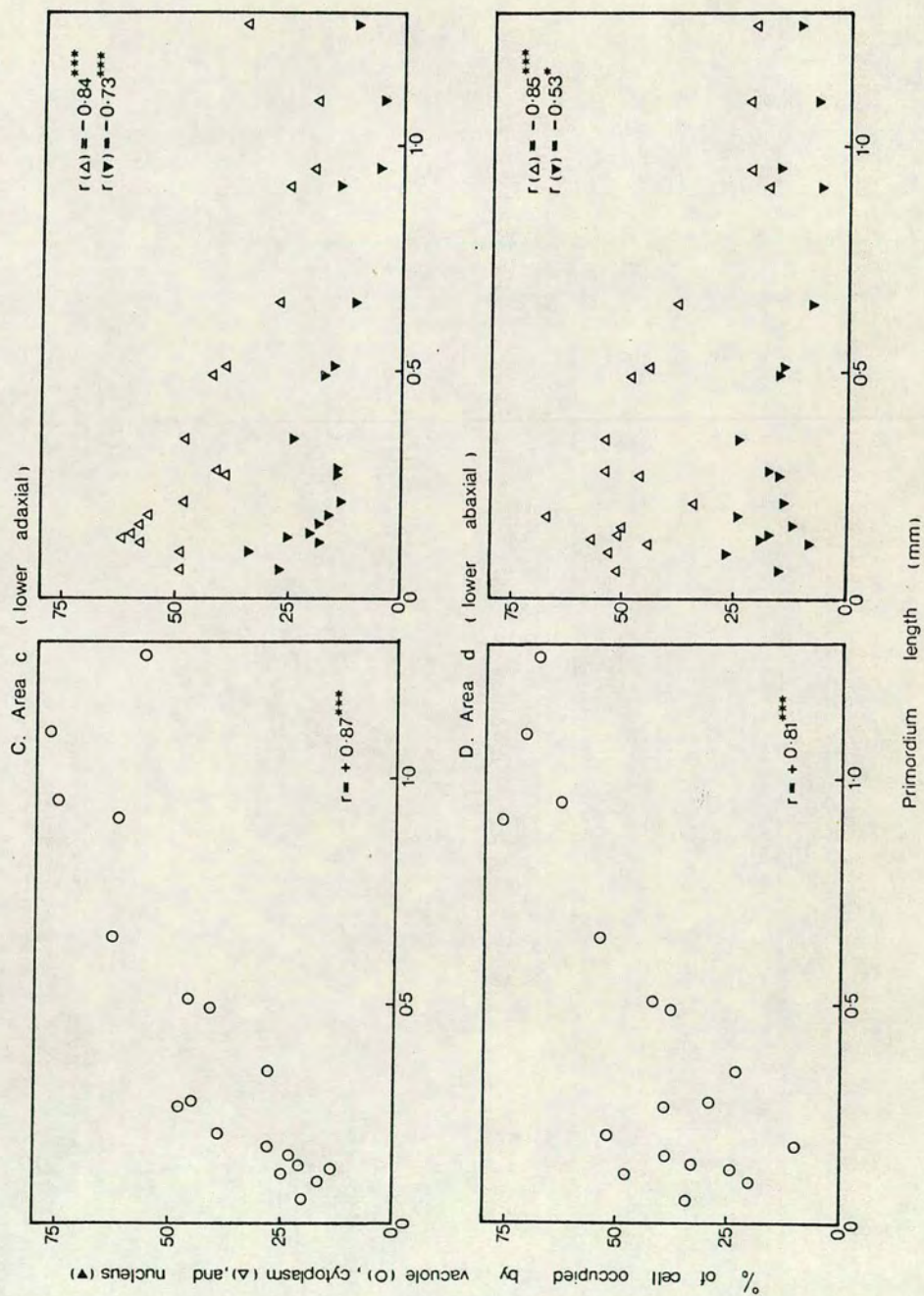




Figure 43. Illustrations of the changes in the proportion of the cells occupied by vacuole, cytoplasm and nucleus, with increasing primordium length, in areas a-d. The length of the primordium is given at the top of each column. These primordia are the same as those illustrated in Figure 41.



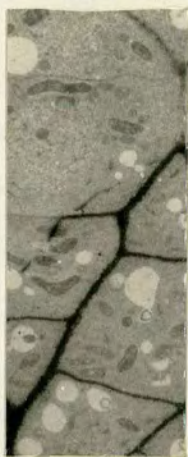
Primordium length (mm)

0.21

0.91

1.27

Area a

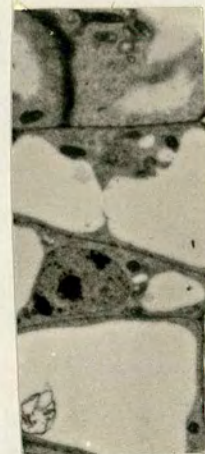
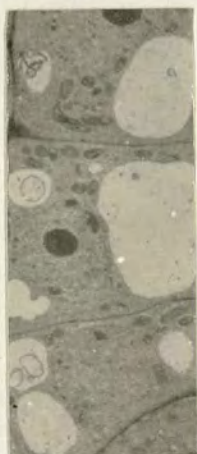


7  $\mu$ m

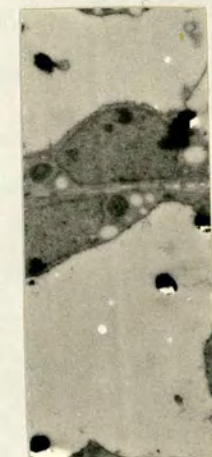
Area b



Area c



Area d





greater than about 0.2 mm long, although in area b (Upper abaxial) they appeared to take place from a very early stage (Fig. 42B). In areas b, c and d, a significant negative correlation between the percentage of the cell occupied by the nucleus and primordium length was obtained (Fig. 42 B-D), suggesting that the percentage of the cell occupied by the nucleus decreased during primordium development. In area a this percentage appeared to remain constant (Fig. 42A).

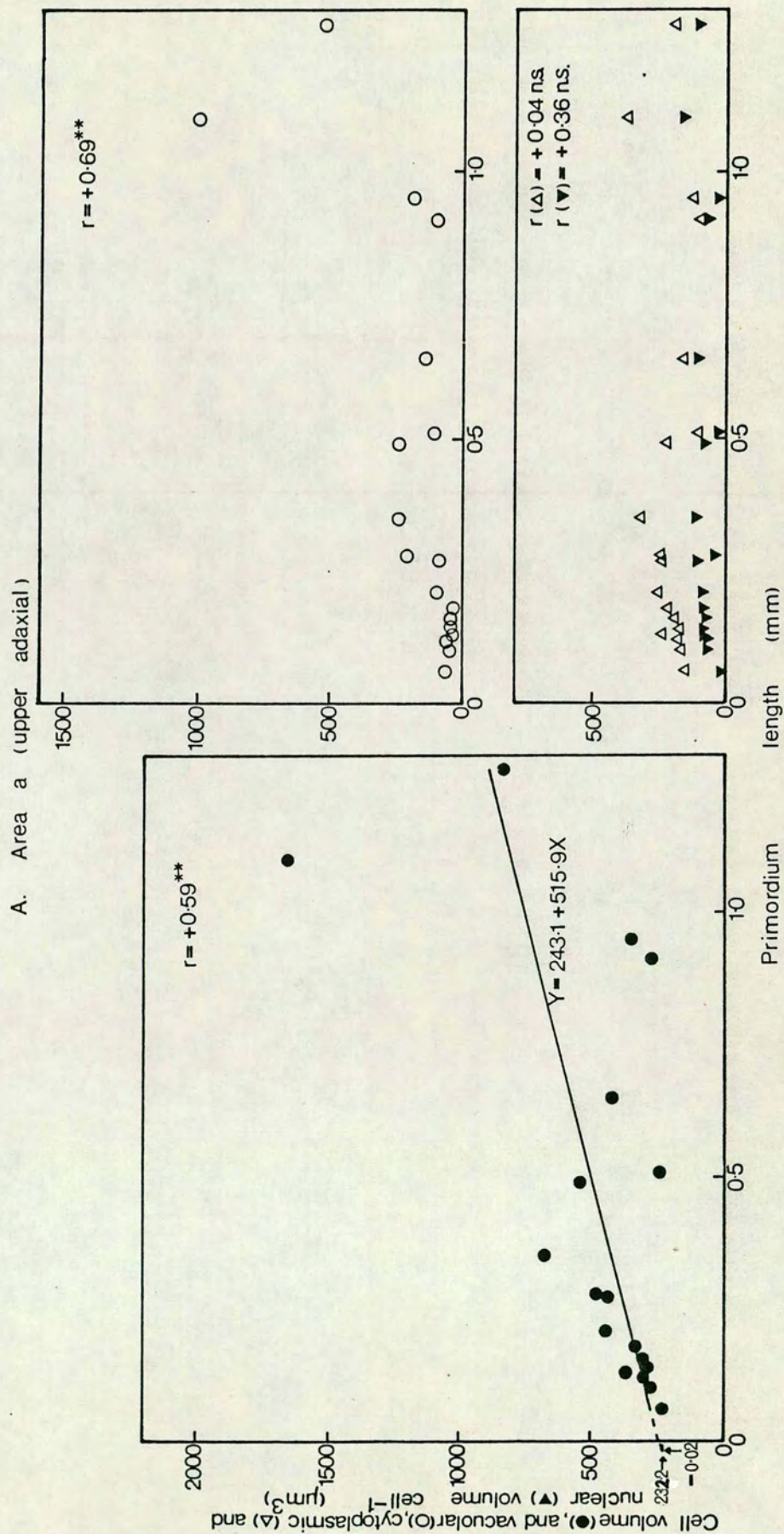
To establish the reason for the increase in the percentage of the cell occupied by vacuole, and the concomitant decrease in the percentage of the cell occupied by cytoplasm, measurements of cell volume were made in each of the four areas. The vacuolar, cytoplasmic and nuclear volumes per cell were then calculated. The results are presented in Figure 44 A-D. In each area significant positive correlations between both cell volume and primordium length, and vacuole volume per cell and primordium length, were obtained. This suggests that cell volume and vacuole volume per cell increased during primordium development. There was no correlation between either cytoplasmic volume per cell and primordium length, or nuclear volume per cell and primordium length, suggesting that the volume of cytoplasm and nucleus per cell remained approximately constant during the phase of primordium development measured.

The implication is that as the primordia grew from about 0.1 to 1.3 mm in length both cell volume and vacuolar volume per cell increased. Since cytoplasmic

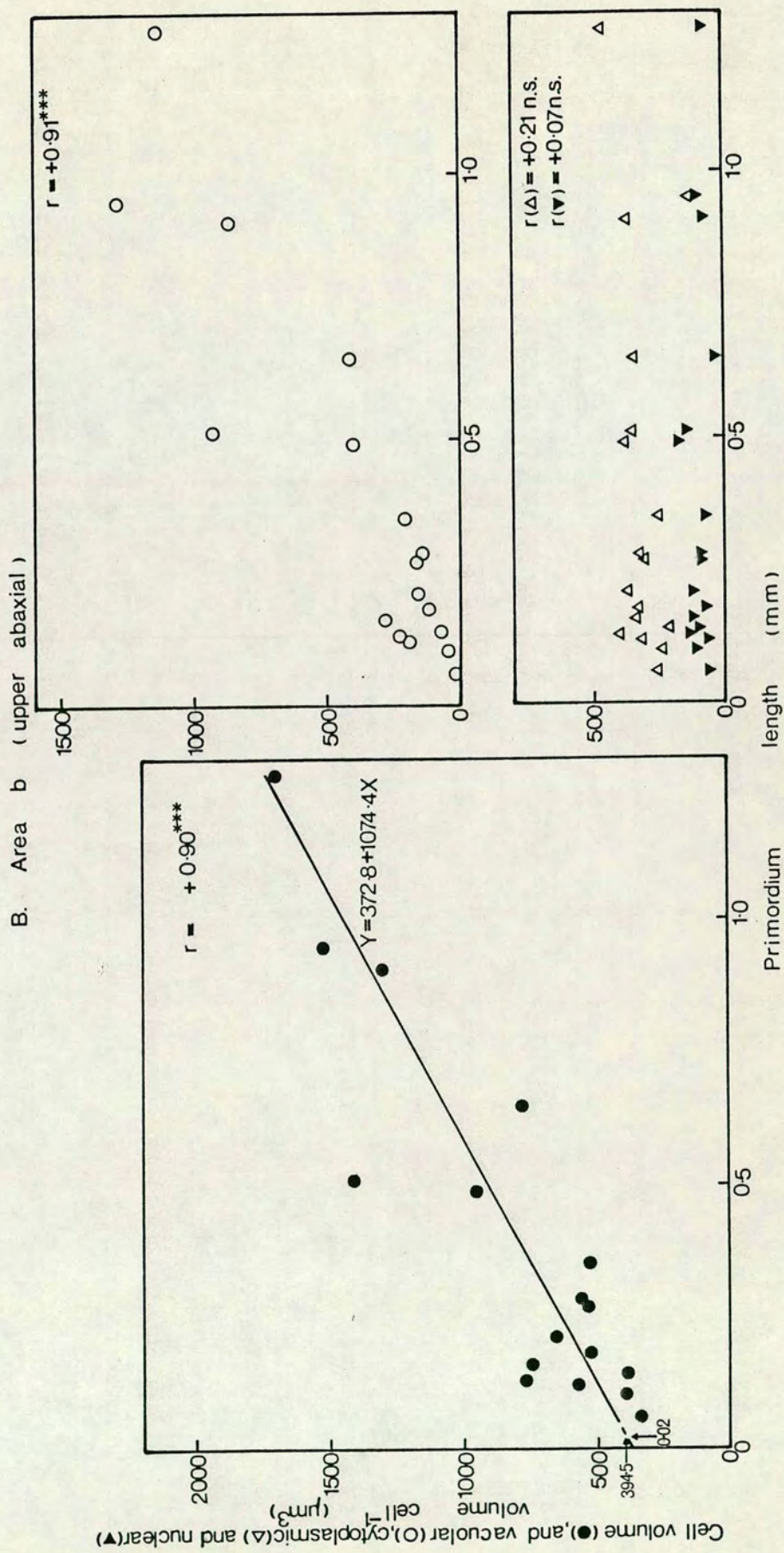


Figure 44. Changes in the volume of the cell (●), and in the volume of vacuole (○), cytoplasm (Δ) and nucleus (▼) per cell, with increasing primordium length, in cells from areas a(A), b(B), c(C) and d(D). The correlation coefficients (r), with the level of significance, are given for each parameter in each area. Regression lines have been fitted to the graphs of cell volume against primordium length and extrapolated back to the cell volume at which vacuolar volume per cell = 0 (see Fig. 45A-D) (horizontally arrowed). The length of the primordium when vacuolation begins can then be read from the graphs (vertically arrowed).  
\*\*\*, \*\*, significant at  $P=0.001$  and  $0.01$ , respectively.  
n.s. - not significant.

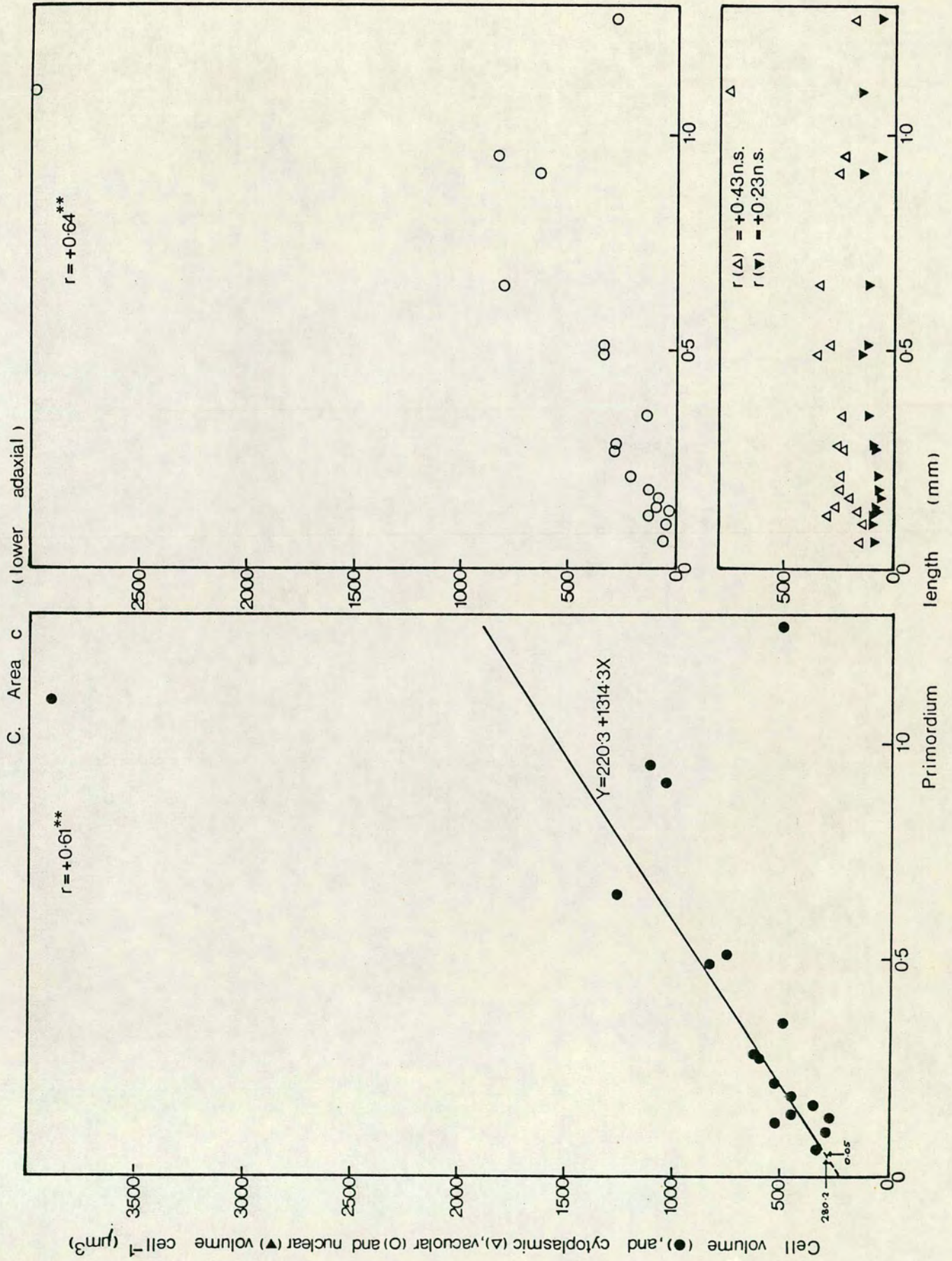




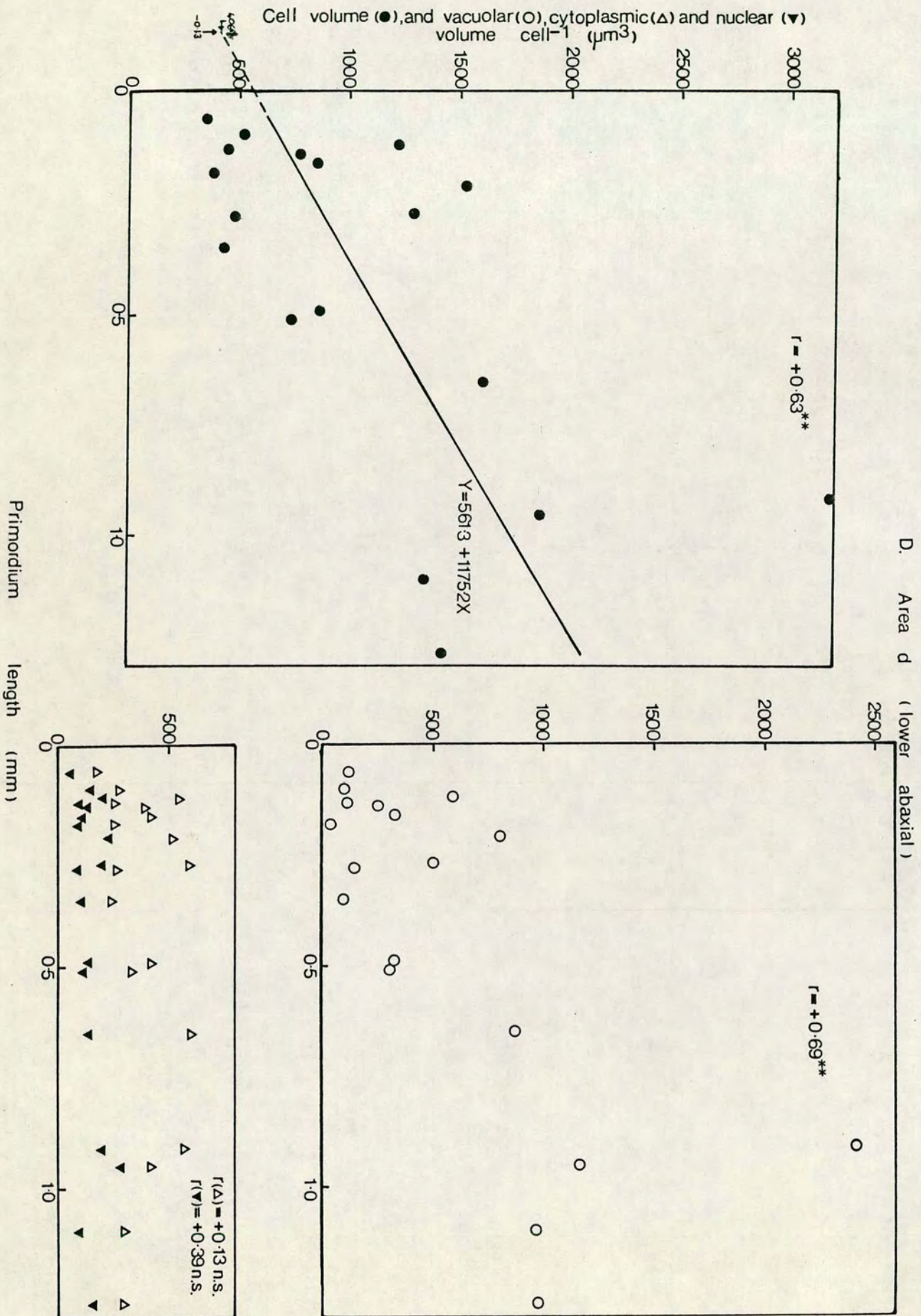














and nuclear volumes per cell remained roughly constant, the cells increased in volume as a result of the increase in vacuolar volume. This conclusion is supported by the very good correlation between cell volume and vacuolar volume (Fig. 45 A-D). As can be seen in this figure, the cell volume before vacuolation began (intercept of regression line with ordinate; arrowed in Fig. 45 A-D) was between 230 and 410  $\mu\text{m}^3$ . This was, in each of the four areas, about the volume of the cells at the time of primordium initiation (Fig. 44 A-D). This suggests that vacuolation began at about the time that the primordia were initiated, and that cell volume and vacuole volume per cell increased in step as the primordium elongated from 0 to about 1.3 mm. Furthermore, the fact that the regression coefficients to the fitted lines in Fig. 45 A-D are all equal to, or just above, unity, suggests that most of the increase in cell volume that took place is accounted for by a proportionate increase in vacuolar volume per cell.

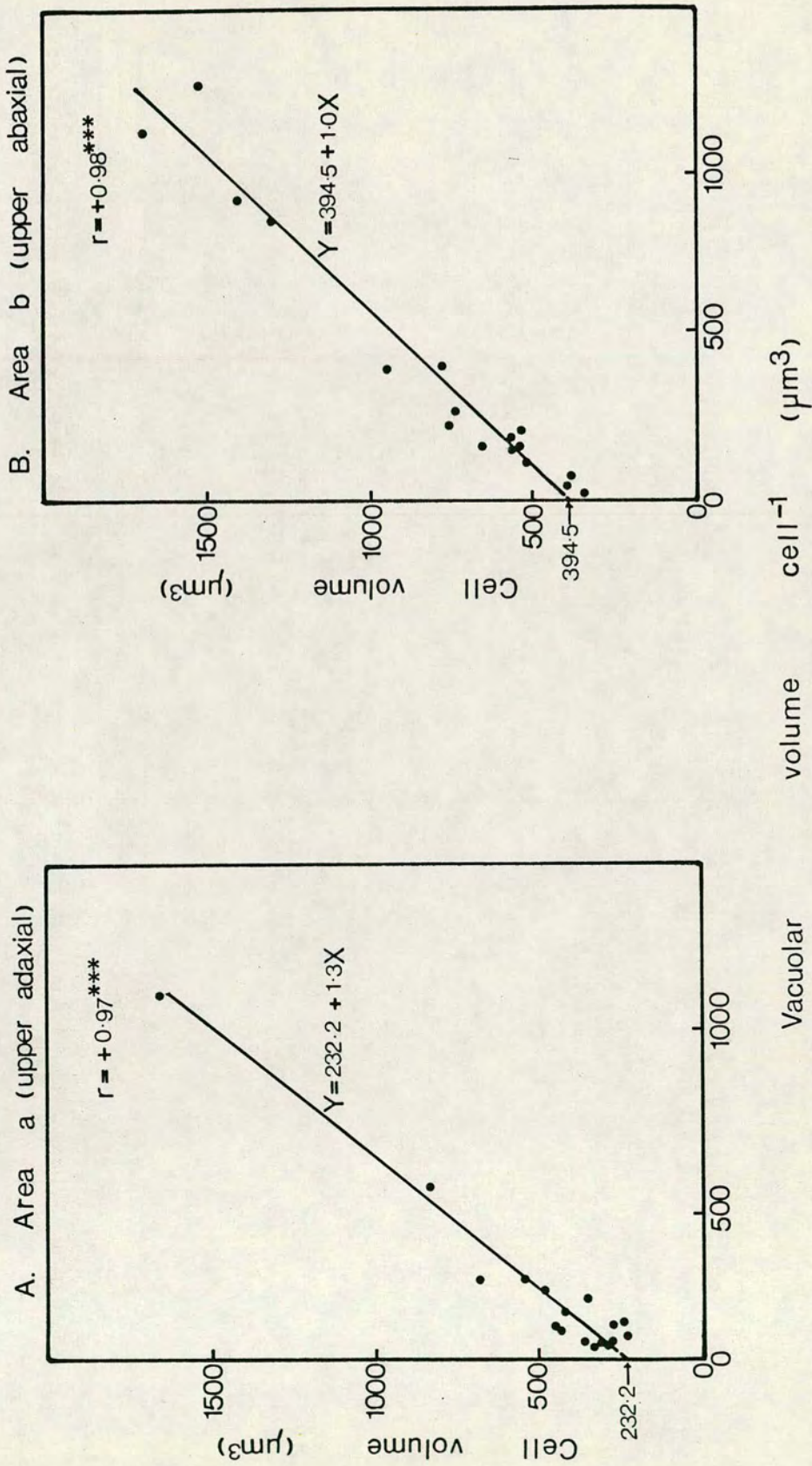
It could be argued that even the limited extrapolation of the regression lines back to 0 vacuolar volume per cell (Fig. 45 A-D) is unjustified, on the grounds that, in other species such as the pea, the apical dome and leaf buttress have vacuoles (Lyndon and Robertson, 1976). However, the average vacuolar volume per cell is about 2  $\mu\text{m}^3$  in the pea leaf buttress (Fig. 5, Lyndon and Robertson, 1976), which is negligible compared with the vacuolar volumes measured during primordium development here. Also, the cell volume at primordium initiation in Impatiens



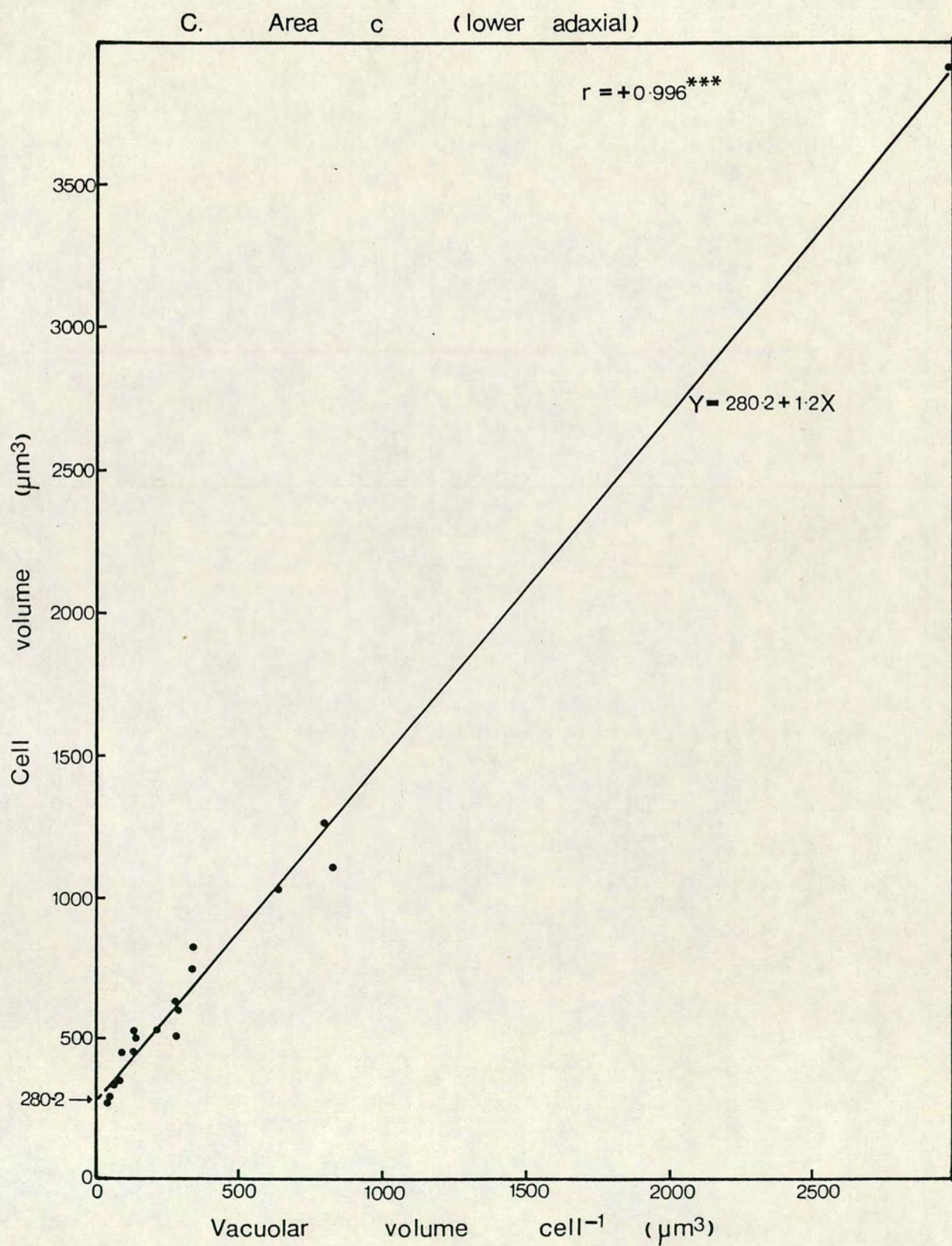
Figure 45. Correlation between cell volume and vacuolar volume per cell, in areas a (A), b (B), c (C), and d (D). The correlation coefficient, and the level of significance, is given in each case. The fitted regression lines and the cell volume when vacuolar volume per cell = 0 (arrowed) are also shown.

\*\*\*, significant at  $P=0.001$ .

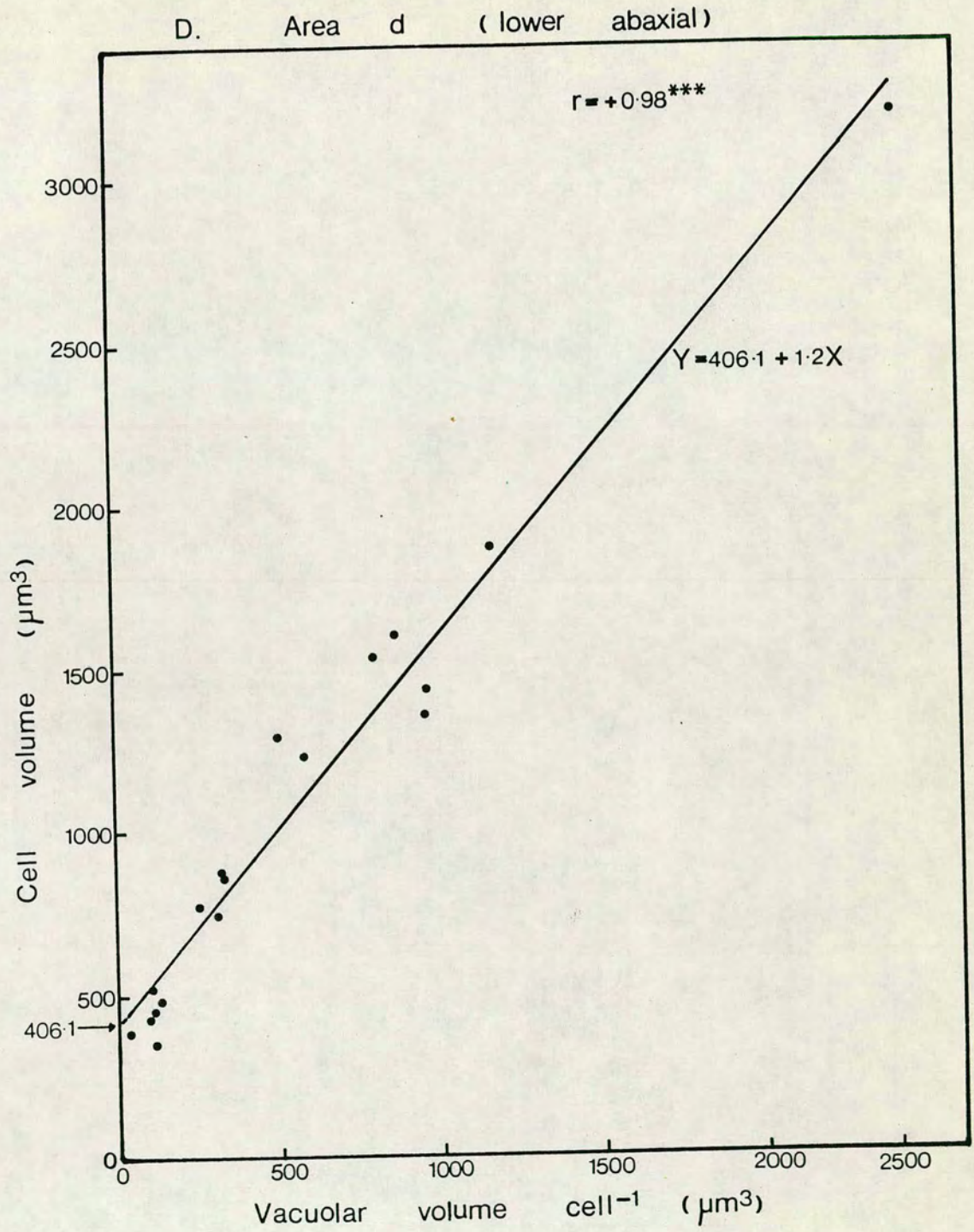














estimated by this extrapolation (about  $230-410 \mu\text{m}^3$ ; see Fig. 45 A-D) is roughly comparable with that found in the pea leaf buttress ( $450-500 \mu\text{m}^3$ ; see Fig 7, Lyndon and Robertson, 1976). It therefore seems reasonable to conclude that vacuolation proceeds steadily from the time of primordium initiation until the <sup>primordium</sup> is 1.3 mm long, in Impatiens.

This process of cell vacuolation roughly paralleled the process of primordium commitment, as measured by the percentage area of the surface occupied by petal pigment in parts 32-34 at maturity (Fig. 39A). However, there is no suggestion that areas a and b, which typically became committed to leaf development earlier than areas c and d (Fig. 40), vacuolated earlier than areas c and d. Indeed, in area a vacuolation appears to have taken place slightly later than in the other areas (Fig. 44).

The conclusion is therefore that commitment to leaf development occurs at about the same time as cells of the primordium mesophyll undergo vacuolation. To test whether this relationship is actually a causal one (vacuolation in some way rendering cells incapable of responding to SD stimuli), further experiments, designed to either delay or accelerate the process of cell vacuolation, would be needed. It could then be established whether commitment is either delayed or hastened as a consequence.

The stereological analysis at level 2 (see Section 2.7, p.29 ), was designed to measure the number of plastids per cell, and the average volume of these plastids, in primordia (parts 32-34) of increasing length. The results



are presented in Figures 46 and 47; the appearance of the cells in each area at this level is illustrated in Figure 48 A-C. Although there was considerable variation, the number of plastids per cell appeared to remain approximately constant, at about 50-60, as the primordia increase from about 0.1 to 1.3 mm in length. This value is in good agreement with the data of Possingham and Saurer (1969) and Boffey et al (1979), who both found about 50 plastids per cell in the earliest stages of development measured by them. Plastid volume also remained constant, at about  $0.3-0.6 \mu\text{m}^3$ , in each of the four areas (Fig. 47 A-D).

The conclusion is that the expected increase in the number of plastids per cell, and the increase in plastid volume that might be expected as the plastids matured and eventually became photosynthetically active, did not occur until the primordia were greater than about 1.3 mm long. Thus commitment of cells of the primordia as leaf cells was clearly not a consequence of an increase in the number of plastids per cell, or in the volume of the plastids. This suggests that such an increase, when it occurs, may be a consequence of the commitment step; it certainly did not cause commitment.

In summary, the findings of this section are that the primordia on the reverted apex of Impatiens became committed to leaf development as their length increased from 0 to 750  $\mu\text{m}$ . After commitment the primordia could no longer develop as petals if the plants were transferred back to SD. Commitment generally occurred first in the tip, and last in the base, of the primordia. Stereological



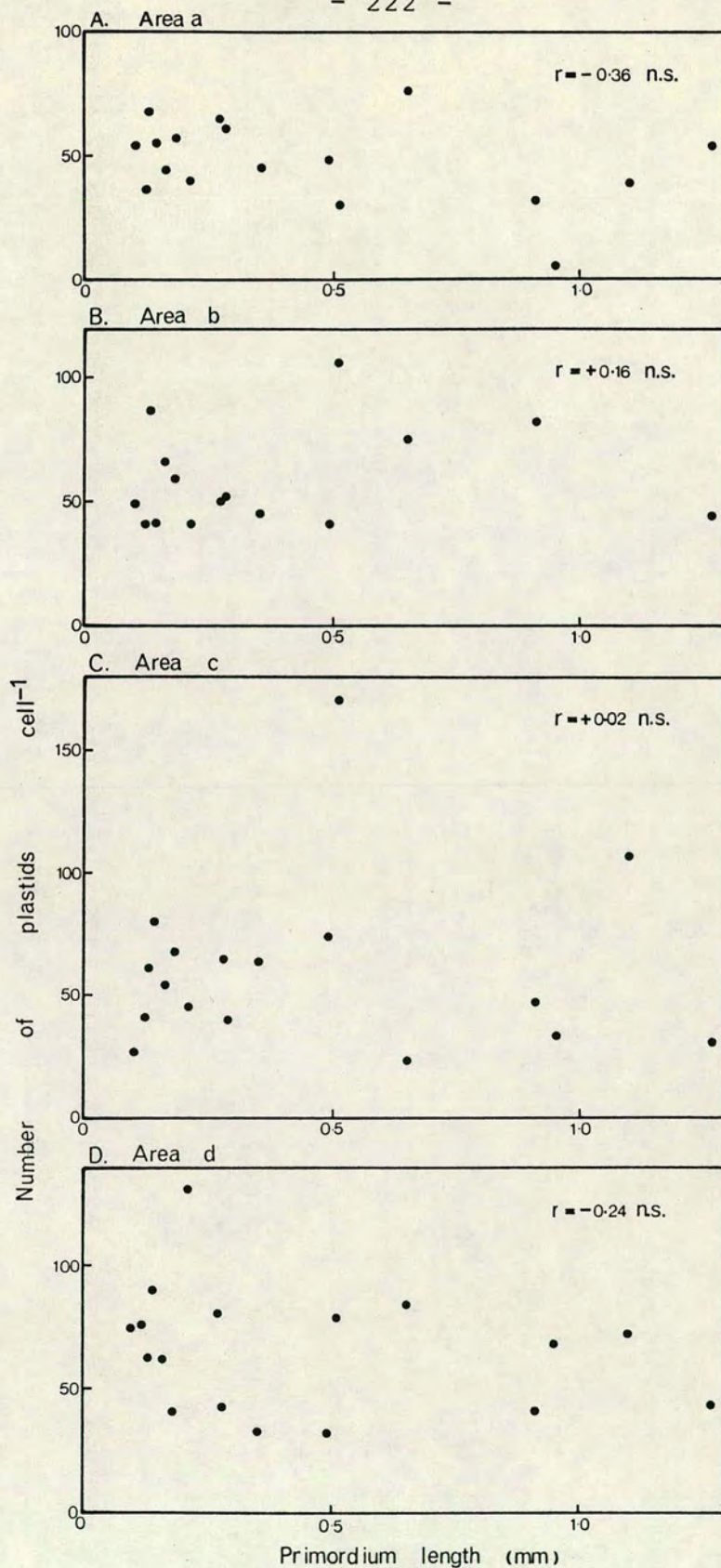


Figure 46. Changes in the number of plastids per cell, with increasing primordium length, in areas a(A), b(B), c(C), and d(D). The correlation coefficient (r), with the level of significance, is given in each case. n.s. - not significant.



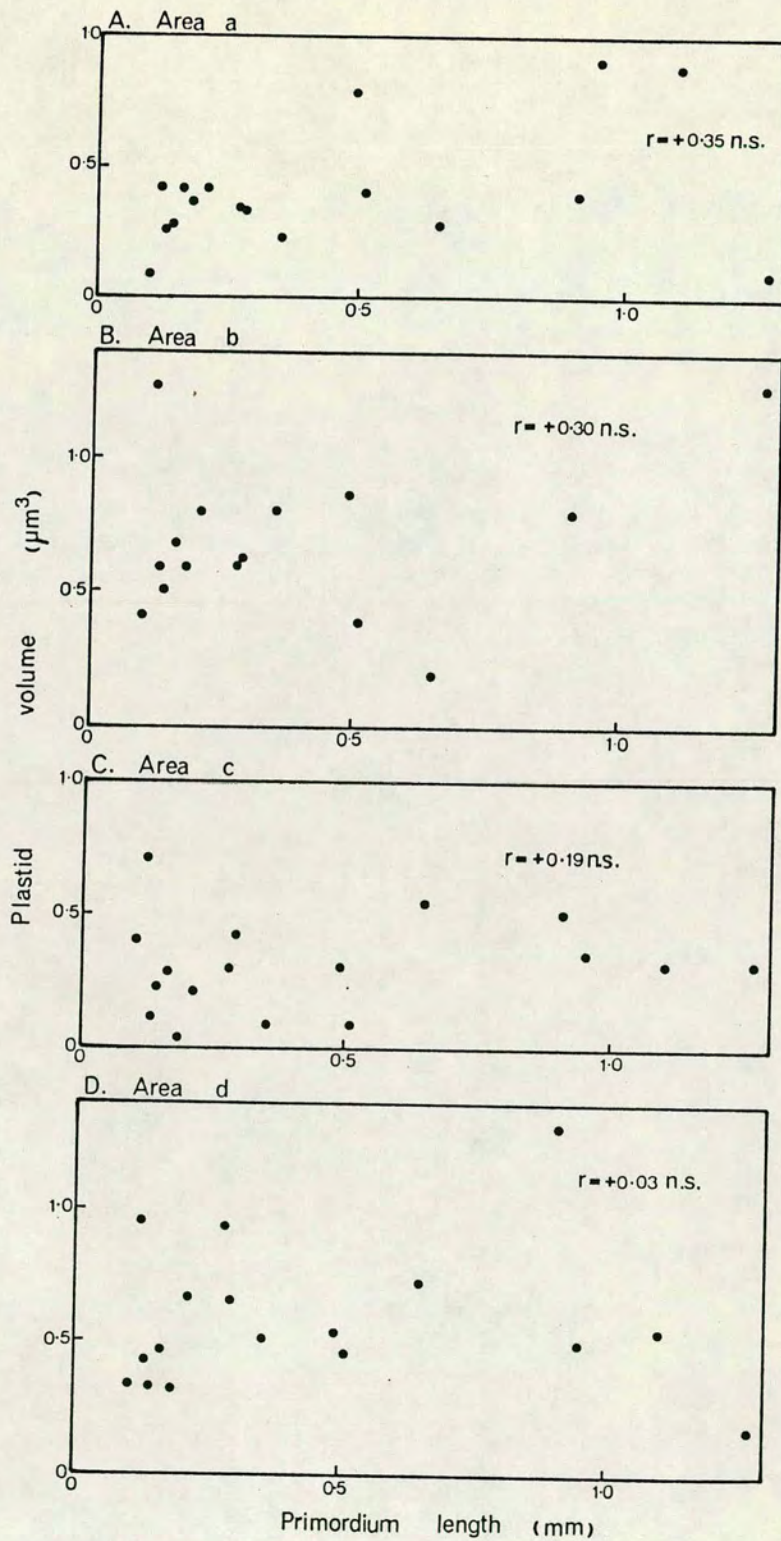


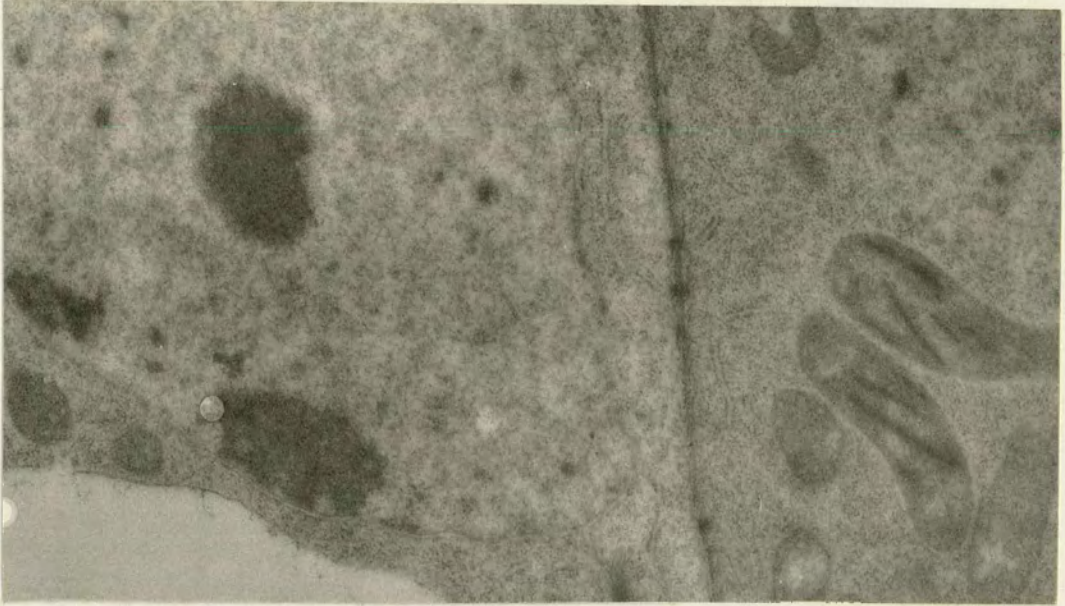
Figure 47. Changes in plastid volume with increasing primordium length, in areas a(A), b(B), c(C), and d(D). The correlation coefficient (r), with the level of significance, is given in each case. n.s. - not significant.



Figure 48. Illustrations of the numbers of plastids in representative area b of primordia of increasing length. The primordia are 0.21 (A), 0.91 (B), and 1.27 (C) mm long, and are the same as those illustrated in Figures 41 and 43.



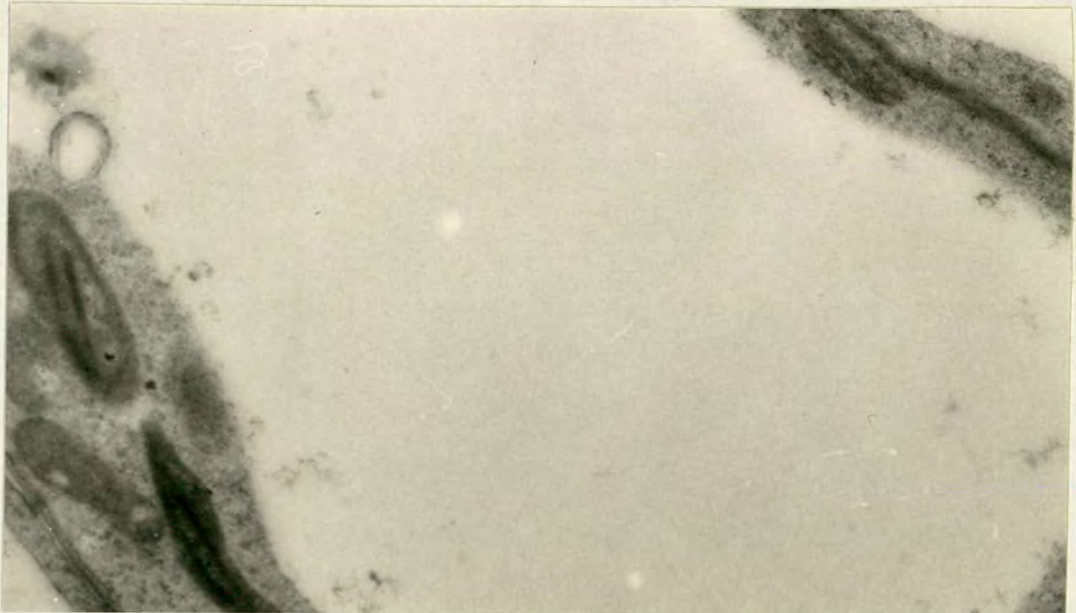
A



B



C





analysis of the sub-epidermal cells of the primordia shows that during the time that commitment took place, vacuolation of the cells also occurred, causing cell volume to increase. There were no changes in the numbers of plastids per cell, or in the average volume of the plastids, during the time that commitment occurred. It would be of interest to explore the relationship between vacuolation and commitment further, to test whether the two processes are linked. If they are, then it may be possible to establish the way in which vacuolation renders cells incapable of responding to SD stimuli. An understanding of the mechanism behind primordium commitment might thus shed light on the nature of the floral stimuli themselves.



CHAPTER 7  
DISCUSSION



## CHAPTER 7: DISCUSSION

### 7.1 Changes in apical growth and phyllotaxis on flowering, reversion and re-flowering

#### 7.1.1 Are evocational changes in apical growth and phyllotaxis essential for flower part initiation?

None of the changes in apical growth and phyllotaxis that occurred on evocation of Impatiens balsamina was completely reversed on reversion (Section 4, p. 69). Furthermore, on re-flowering of the reverted apex, no changes in apical growth and phyllotaxis were found (Section 5, p. 119). Together, these data indicate that changes in growth and phyllotaxis are not necessarily associated with the transition to and from flower part initiation. However, the attainment of a mode of apical growth and phyllotaxis different from that which sustains vegetative growth may be an essential prerequisite for flowering in Impatiens. This is consistent with the fact that, in other species in which measurements have been made, a new mode of apical growth and phyllotaxis is associated with flower formation (Lyndon, 1978; Meicenheimer, 1979). The extent to which the flowering apex differs from the vegetative apex varies according to the parameter measured and the species studied (Table 40). However, in the flower of each species the rate of primordium initiation is higher, and the absolute area of the primordium on initiation smaller, than in the vegetative apex. If the rate of primordium initiation is the resultant of the apical growth rate and the area of the primordia initiated



TABLE 40.

Comparison of apical cross-sectional area, apical growth rate, absolute primordium area at initiation, and rate of primordium initiation in flowering apices of three species for which measurements are available. Parameters are shown as higher (+), the same as (0), or lower (-) than in the vegetative apex; calculated values (relative to the vegetative apex = 10) are shown in parenthesis

Species (Reference)	Organ type	Cross sectional area of apex	Apical growth rate	Absolute primordium area at initiation (corrected for api- cal shape)	Rate of primordium initiation
* <u>S. coeli-rosa</u> (Lyndon, 1978; 1979a)	Sepals	+ (17)	+ (16)	- (4)	+ (98)
	Petals	+ (18)	?	- (2)	+ (195)
	Stamens	+ (11)	- (6)	- (2)	+ (53)
* <u>R. repens</u> (Meichenheimer, 1979)	Sepals	+ (14)	0 (8)	- (4)	+ (23)
	Petals	+ (30)	0 (?)	- (?)	+ (230)
	Stamens	+ (33)	0 (8)	- (1)	+ (230)
* <u>I. balsamina</u> (Tables 12,15, 16, 21, 22, 30) (Transfer to SD, day 0)	Bracts/ petals	0 (11)	?-increasing	0 (13)	?-increasing
	Petals	0 (7)	+ (20-37)	- (4)	+ (42)
	Stamens/ Carpels	0 (6)	+ (19-27)	- (4)	+ (14-28)

\* Silene coeli-rosa\*\* Ranunculus repens\*\*\* Impatiens balsamina



by the apex (Richards, 1951), then the crucial difference between the flowering and the vegetative apex may be that the absolute area of the primordium at initiation is smaller in the flowering apex (see Table 40). A decrease in the area of the primordium at initiation could be an important factor determining the change in phyllotaxis that occurs in Silene coeli-rosa, Ranunculus repens and Impatiens on flowering (Lyndon, 1978; Meicenheimer, 1979; Section 4.2.2, p. 80), since an increase in the area of the apex relative to that of the primordium on initiation would be expected to cause a shift to higher-order phyllotaxis (Richards, 1951).

Therefore, the attainment of a new mode of apical growth and phyllotaxis, in which the absolute primordium area at initiation is smaller than during vegetative growth, may be a prerequisite for the development of organs as flower parts instead of as leaves. If this is so, and the particular growth changes that occur during the transition to flowering are not in themselves essential to flower part initiation, then these growth changes might be expected to differ in different species; this seems to be the case. In Silene the growth rate increases during the transition to flowering (Miller and Lyndon, 1976), but it declines during subsequent flower formation (Lyndon, 1979a; see Table 40). On the other hand, in Ranunculus the growth rate remains constant during the transition to flowering, whilst in Impatiens it increases during petal initiation, and remains higher than in the vegetative apex during subsequent flower formation



(Tables 21 and 22, p.113 and 114). Similarly, whilst the apex increases in cross-sectional area on flowering in Silene (Lyndon, 1978) and Ranunculus (Meicenheimer, 1979), its area remains constant in Impatiens plants transferred to SD on day 0 (Table 15, p.87 ). Thus the modified phyllotaxis of the flower is achieved in different ways in different species; it may also be achieved in different ways within a single species, as is the case in Silene induced at different temperatures (Lyndon, 1977, 1979a) and Impatiens transferred to SD on day 0 and day 29 (compare Tables 15 and 30, p.<sup>87</sup> & 147). In Impatiens, an increase in apical area occurred during the Early petal stage in plants transferred to SD on day 29, but not in those transferred to SD on day 0 (Tables 30 and 15, p.147 and p.87 ).

Therefore, although variations can occur in the way in which the phyllotaxis of the flower is attained, a consistent (possibly essential) feature of all flowers measured is a decreased absolute primordium area at initiation, compared with that in the vegetative apex. The variation in the way floral phyllotaxis is attained also suggests that the lack of increase in apical area on flowering of Impatiens plants transferred to SD on day 0 may not be linked to the ability of these plants to revert. The marked increase in apical size during the transition to flowering of most species (see Table 1, p. 5 ) may merely be a particularly common way of attaining the higher-order phyllotaxis typically associated with flowering. Furthermore, in both Humulus lup<sup>ul</sup>ulus (Thomas and Schwabe,



1970) and Perilla nankinensis (red-leaved) Nougarede et al., 1964) the apex decreases in size on flowering, and yet only Perilla has been reported to show reversion (Nougarede et al., 1964). However, the idea that a constant apical area on flowering may be linked to reversion could be tested by returning Impatiens plants transferred to SD on day 29 to LD, and seeing if reversion occurs. If the transient increase in apical area at the Early petal stage of flowering in these plants is significant, it should prevent their reversion to vegetative growth on return to LD. Similarly, in other species showing flower reversion (e.g. Anagallis arvensis and Pharbitis nil), future work could determine whether apical area remains constant on flowering.

In conclusion: it seems that the way in which the new phyllotaxis associated with flower part initiation is attained may not be critical. One function of evocation may therefore be to achieve a new primordium arrangement, which may, in turn, allow the initiation of flower parts.

7.1.2 If the reverted apex is partially evoked, are the leaves of the reverted plant also partially induced?

Although in Impatiens the modified primordium arrangement of the flower may have been essential for flower part initiation, it did not preclude leaf initiation, since on reversion primordium area at initiation, and arrangement, remained the same as in the flower for at least 15 days after the resumption of leaf initiation (Section 4.2, p.74 ). Therefore Impatiens can have two types of vegetative apex,



each with a characteristic primordium arrangement, rate of primordium initiation and apical growth. The rates of primordium initiation and apical growth both decreased relative to those in the flowering apex, on reversion (Section 4.2, p. 74). However, this does not appear to have constituted a process of 'de-evocation', because on re-transfer of reverted plants to SD, petal initiation began again immediately and no changes in growth and phyllotaxis were observed (Section 5.1, p.125). The implication of this could be that, although Impatiens did not become committed to flowering, changes occurred during evocation that were not immediately dissipated on return to LD. This is also suggested by the fact that no bracts were initiated on return to SD, as they were during flowering of vegetative (LD) plants transferred to SD. The reverted apex could therefore be described as partially evoked, meaning that some of the changes necessary for the initiation of flower parts have been achieved (Bernier et al., 1981b). This implies that the metabolic changes associated with flower part initiation can be separated from those controlling primordium arrangement. This may also happen in Xanthium pennsylvanicum (= X. strumarium), in which gibberellic acid treatment caused the phyllotaxis to change from 2:3 to a 3:5 contact parastichy arrangement (Maksymowych and Erickson, 1977). This is a similar change to that which occurs at the transition to flowering/ <sup>in Xanthium</sup> (Erickson and Meicenheimer, 1977), but flowering was not induced by treatment with gibberellic acid. However, reverted



Impatiens plants were also more reactive to inductive conditions than vegetative (LD) plants (Section 5.1, p.125), whereas this was not demonstrated in Xanthium. The increased reactivity of the reverted apex was not due to physiological ageing of the apex and/or the leaves, since vegetative plants transferred to SD on day 29 were less reactive than reverted plants (Section 5.1, p.125).

Although the reverted apex was partially evoked, the increased reactivity of reverted plants to SD could have resulted from changes that occurred in the apex, or in the leaves, in response to the 5SD treatment. If more than one substance, or one substance evoking the successive steps in flowering at different concentrations in the apex, is produced by the leaves of Impatiens, then the 5SD treatment might cause the leaves to become partially induced. In this case, initial transfer to SD might cause a stimulus inducing changes in apical growth and phyllotaxis to be translocated to the apex. After about 5SD, a second stimulus, causing petal formation, might reach the apex; transfer to LD at this time would prevent further translocation of the second stimulus, so that leaf production resumed at the apex, with the primordia arranged as they were in the flower. When reverted plants were returned to SD, the leaves would immediately begin to produce the stimulus causing petal formation again. On this hypothesis, the leaves of the reverted plant would be partially induced. On the other hand, if the leaves only produced a single stimulus to flower,



then the increased reactivity of reverted plants to SD would be determined by the apex. The fact that the reverted apex had achieved a mode of growth and phyllotaxis very similar to that in the flower suggests that this may be so. At present, the question of whether the increased reactivity of the reverted plants results from changes occurring in the leaves or the apex cannot be resolved. Experiments to distinguish between the two possibilities are suggested in Section 7.3 (p. 282).

Eventually the reverted apex returned to a mode of apical growth and phyllotaxis typical of the vegetative (LD) apex (Section 4.2, p. 74). This implies that the reverted apex did not remain partially evoked indefinitely, and that the metabolic changes that occurred on evocation of the apex were eventually reversed completely. It is not known whether, after complete reversion, the response to SD returned to that of the original vegetative (LD) apex. This might be expected, however, given the previous correlation between the mode of apical growth and phyllotaxis, and the response to SD (see above).

7.1.3 How are the changes in phyllotaxis and apical growth controlled?

A. The change from spiral to whorled or pseudo-whorled phyllotaxis: a decrease in divergence angle?

In the vegetative apex the sequence in which primordia arose was obviously regular, with a divergence angle of about  $137^{\circ}$  between successive primordia (Section



4.2.2, p.80 ). During the transition to flowering this genetic spiral could be followed until about part 20. After this, primordia were initiated in whorls or pseudo-whorls, but the order of initiation became difficult to establish (Section 2.6.4, p. 24). For practical purposes it was therefore assumed that the relative radial distances of primordia reflected their order of initiation on flowering and reverting apices. However, it is reasonable to ask what the true divergence angle was in the flowering and reverting apices. Some tentative answers can be obtained by examining the four apices illustrated in Figure 49.

In the apex illustrated in Figure 49A the genetic spiral becomes difficult to follow after about part 16; the arrangement from part 18 (labelled 1' on Figure 49A) onwards is best described as a pseudo-whorl of five primordia, followed by two pseudo-whorls of six, and then two more regular whorls of five. If an approximately spiral order of initiation is assumed, then the divergence angle alters from about  $137^{\circ}$  in the vegetative apex to an average of  $132^{\circ}$  in whorls of six, and  $144^{\circ}$  in whorls of five primordia. Thus an overall average divergence angle of  $139^{\circ}$  would occur after the attainment of pseudo-whorled or whorled phyllotaxis, with contact parastichies of 5:5 or 6:6, although these are irregular because of the varying numbers of primordia in a whorl. However, the evidence for an approximately spiral order of initiation in whorled or pseudo-whorled apices of Impatiens is often not good. For instance, in the apex illustrated in Figure



Figure 49.

A) Camera lucida drawing of the transverse section just above the apical surface of a flowering apex sampled after 17SD. Parts 10 to 16 numbered in sequence with approx.  $137^{\circ}$  divergence angle. Parts 18 (1') onwards numbered in whorls or pseudo-whorls 1 to 5; roman numeral superscripts indicate component members of each whorl or pseudo-whorl. Contact parastichies indicated by full or broken lines linking the centres of primordia in successive whorls or pseudo-whorls.

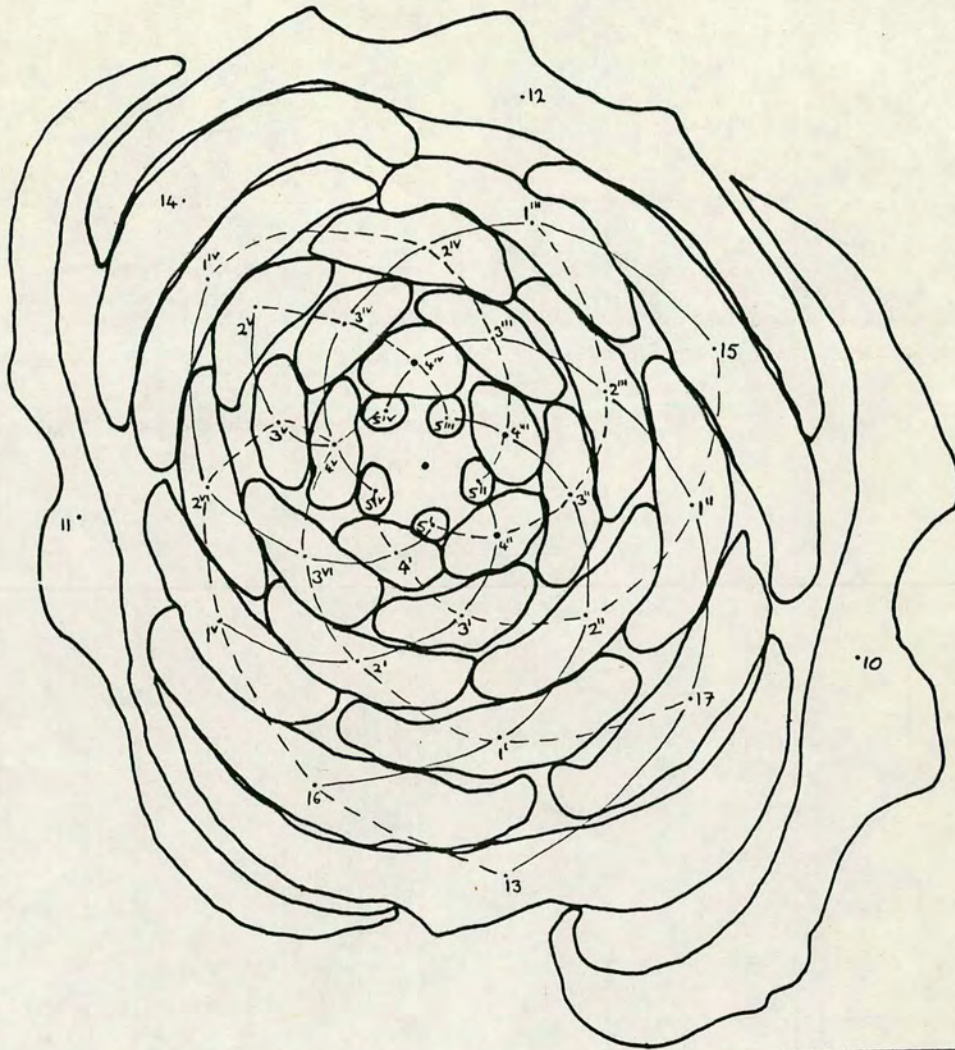
B) Tracing from an S E M photograph of the shoot apex of a reverted plant sampled after 5SD+7LD. Primordia of youngest pseudo-whorl numbered according to relative areas occupied (1=smallest area occupied). See also Figure 12C, p.

C) Tracing from a photograph of the transverse section just above the apical surface of a reverted apex sampled after 5SD+17LD. Parts 22 to 27 numbered with very approx.  $137^{\circ}$  divergence angle between successive primordia. Parts 28 onwards numbered according to approx. radial distance from the centre of the apex. Irregularities between parts 28 and 41 have been assumed to result from changes in the relative positions of primordia during their displacement from the apical centre. 6:7 contact parastichies indicated by full and broken lines linking the centres of primordia. Orthostichies indicated by dotted lines.

D) Tracing from a photograph of the transverse section just above the apical surface of a reverted apex sampled after 5SD+9LD. Parts 13 to 34 numbered with very approx.  $137^{\circ}$  divergence angle between successive primordia. 3:5 contact parastichies indicated by full and broken lines, and 5:8 contact parastichies indicated by broken and broken crossed lines, linking the centres of primordia.

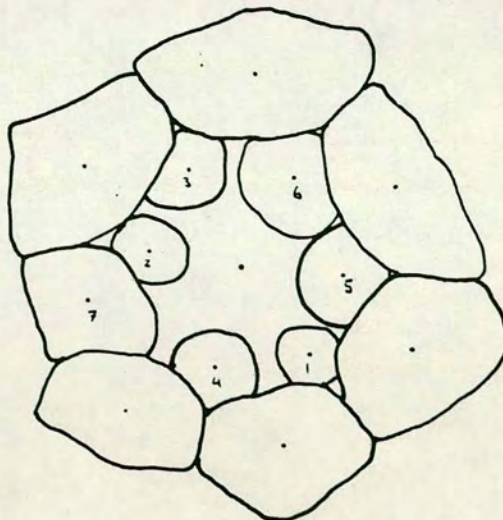


A



500 μm

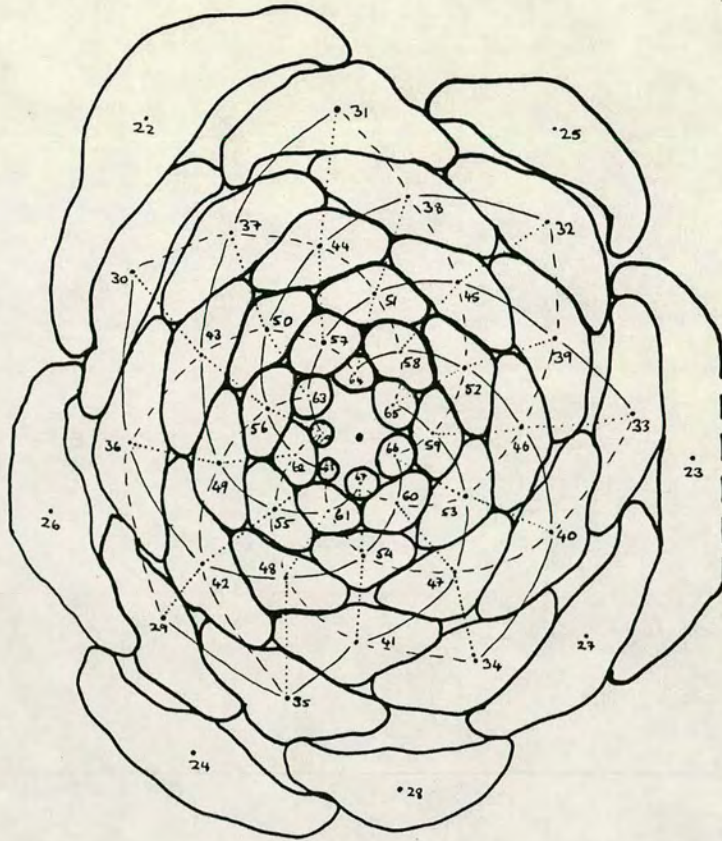
B



100 μm

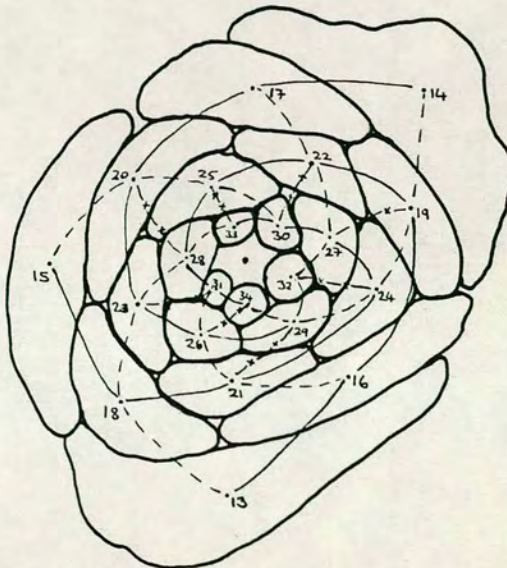


C



500  $\mu$ m

D



500  $\mu$ m



49B, the slight unevenness in the sizes of the members of the youngest pseudo-whorl could result from slight differences in their initiation time. If this is so, then the order of initiation (numbering from the youngest primordium upwards) is not spiral; the divergence angle calculated on this basis is about  $104^{\circ}$ .

However, it may be that in attempting to determine the divergence angle between primordia within a whorl (or pseudo-whorl), the important point about whorled (as opposed to spiral) phyllotaxis is passed over. This point may be that because the primordia within a whorl (or pseudo-whorl) are initiated at approximately the same time (as judged by their relative sizes or their distances from the apical centre), it is the divergence angle between members of successive whorls that is the equivalent parameter to the divergence angle between successive primordia in spiral phyllotaxis. In the apex illustrated in Figure 49A the angular shift between the two successive alternating whorls of five primordia is about  $36^{\circ}$ . If an alternating whorled system is treated as a multijugate derivative of <sup>the</sup> 1:1 alternate system (Richards, 1951; Thomas, 1975), then  $36^{\circ}$  is the divergence angle between successive members of the same genetic spiral. There are, in this case, five genetic spirals winding around the apex during the initiation of these two whorls. In this case, pseudo-whorls would be deviations from an approximately whorled system of phyllotaxis; such deviations might result from slight differences in the absolute size at initiation of the primordia, and/or their time of initiation. The



difference in appearance of the youngest whorls in Figure 49A and B might result from either or both of these causes.

If this interpretation is correct, the divergence angle would be about  $36^{\circ}$  in apices initiating whorls of five primordia, and about  $60^{\circ}$  in apices initiating whorls of six primordia. The possibility of such a small divergence angle is supported by the apex illustrated in Figure 49C, in which true whorls are not present. Apices of this type were noted on several occasions in both flowering and reverting plants. The plant from which the apex illustrated in Figure 49C was sampled had been given 5SD + LD, and was unusual in that it had initiated 69 leaves plus primordia, but had not yet returned to the spiral phyllotaxis typical of vegetative (LD) plants. However, it illustrates well a primordium arrangement in which successively younger primordia (as judged by either radial distance from the centre or size) are initiated with a divergence angle of only about  $55^{\circ}$ . This gives, from about part 29 onwards, a fairly regular 6:7 contact parastichy system, with approximately orthogonal intersection towards the centre of the apex. There are also 13 approximately rectiserial orthostichies, giving a 2/13 arrangement in the terminology of Schimper and Braun (Thomas, 1975).

In Figure 49D a fourth type of apex found in flowering and reverted plants is illustrated. 3:5 contact parastichies can be traced, with some irregularities, almost to the youngest primordium, but the 5:8 contact



parastichies become more prominent towards the centre. A genetic spiral, with a very approximate  $137^{\circ}$  divergence angle, can be followed, and the primordia are numbered according to this sequence. However, there is, both from the relative radial distances of the primordia from the centre, and from their relative sizes, a suggestion that the genetic spiral actually runs through adjacent primordia from part 20 onwards. This is very similar to the more obviously apparent spiral in Figure 49C, but in the apex in Figure 49D the divergence angle is about  $70^{\circ}$ . Therefore this apex illustrates an intermediate stage between the spiral phyllotaxis typical of vegetative apices and the arrangement described for the apex in Figure 49C. There may also be two pseudo-whorls, from part 26-29, and part 30-34, so that this apex also has some of the characteristics of the apex illustrated in Figure 49A.

These examples suggest that, on the transition to whorled or pseudo-whorled phyllotaxis, the divergence angle may decrease from that found in vegetative apices with spiral phyllotaxis. To be certain that this is so, repeated observation, under a dissecting microscope, of a single, defoliated apex of each of the types discussed, would be needed. Although this was not done in the present study, and it might be technically difficult, it has been possible in fern apices (Cutter, 1964). If the interpretation of the apices in Figure 49 is correct, what are the implications? The proposal that in whorls or pseudo-whorls of  $n$  primordia there are  $n$  genetic spirals winding



around the apex has superficial similarities to the idea of foliar helices proposed by Plantefol (1948) and Loiseau (1969). However, it is probably unnecessary to suggest that leaf-generating centres are operating within each of these spirals, since the following argument, based on the same assumptions that underlie the inhibitor theory of <sup>spiral</sup> phyllotaxis (see Schwabe, 1984b, for review), appear adequate to explain the observations.

If it is assumed (a) that primordia cannot arise within about 40-50  $\mu\text{m}$  of the apical centre, because the apex synthesises an inhibitor of primordium initiation (see e.g., Mitchison, 1977), and (b) that each primordium in a whorl produces an inhibitor of primordium initiation, whose effect either decreases with primordium age (Thornley, 1975), or is determined by its predominantly polar transport, with some non-polar apoplastic movement (Schwabe, 1984b), then minima in the inhibitor field would occur midway between the primordia of the youngest whorl as the members of this whorl grow older and are displaced below the apical surface. This would result in an alternating whorled system of phyllotaxis.

Thus the same mechanism (production of an inhibitor of primordium initiation by the apex and primordia) can explain spiral and whorled phyllotaxis. There are two points that follow from this. Firstly, the decreased divergence angle between the members of successive whorls of primordia (compared to that between spirally arranged primordia) is an expression of the fact that in



whorled phyllotaxis several primordia are initiated approximately simultaneously, rather than sequentially. Secondly, it is the transition to and from whorled phyllotaxis, during which the apex changes from the initiation of one primordium, to several primordia, at a node, that must be accounted for. The irregular pseudo-whorls of about four to six primordia (Fig. 49A, B and D) might arise as this change occurs; apices such as that illustrated in Figure 49C may occur when a true whorled arrangement is never attained. These possibilities will be considered further in the next section, in which possible mechanisms behind the change to and from whorled phyllotaxis are considered.

B. Mechanisms involved in the transitions to and from whorled or pseudo-whorled phyllotaxis on flowering and reversion

The role of the number of primordia on the apical surface and stem frustum length at initiation

During the transition to flowering the number of primordia on the apical surface increased from three to about eight, and the length of <sup>the</sup> stem frustum at initiation (both relative to the length of the tissue generating the frustum, and in absolute terms) decreased (Section 4, p.69 ). This would be expected to result in an increase in the persistence of the inhibitory effect of young primordia, if transport of the postulated inhibitor of primordium initiation is polar (Schwabe, 1984b). This in turn would be expected to lead to an overall higher



level of inhibition at the apex. If this occurred, then there might follow a period during which a more or less symmetrical ring of inhibition, above the threshold below which primordium initiation could occur, surrounded the apical surface. After a time, inhibition would fall below the threshold as a result of vertical growth of the apex. This would occur at approximately the same time at several different sites around the perimeter of the bare apex, and would result in the initiation of a pseudo-whorl of primordia, which would be expected to be irregular because of the way in which it arose. This is what was observed in many cases (see Figs 12B, 49A, B and D). After initiation of this pseudo-whorl, the primordia that make it up would inhibit further primordium initiation until sufficient vertical growth had been made by the apex to allow another pseudo-whorl to be initiated. In some cases, with successive initiation of pseudo-whorls, the position at which minima in the inhibitor field arose might become more regularly whorled, but this would not necessarily occur; neither was it always observed.

If this idea is correct, then we would expect that, during the transition to whorled or pseudo-whorled phyllotaxis, a number of primordia would be initiated in approximately spiral arrangement before the transition actually occurred, as the number of primordia on the apical surface (and hence the inhibitor level at the apex) built up. This was observed - part 10 to about part 20 were usually in spiral arrangement. Another corollary of this idea is that primordium initiation would be expected to occur in bursts



after the transition to whorled or pseudo-whorled phyllotaxis. This is strongly implied merely by the fact that the arrangement of the primordia was whorled or pseudo-whorled. However, a marked delay might also be expected before the initiation of the first pseudo-whorl of primordia. This was not detected, although it might actually have occurred, since the rates of primordium initiation were obtained as averages for a number of apices, rather than from individual apices studied over a period of time. However, such a delay need not necessarily occur, if it is proposed that the rate of production of inhibitor by the primordia was proportional to their absolute size at initiation. If this were so, then when the primordia became smaller at initiation on flowering (Section 4.2.3, p.86 ), although the symmetrical inhibitor field around the perimeter of the apex would still have occurred as postulated above, less time would have elapsed before the inhibitor fell below the threshold level and allowed the initiation of the first pseudo-whorl of primordia. This may seem a rather ad hoc hypothesis. However, it also allows an explanation of the type of primordium arrangement illustrated in Figure 49C, in which minima in the inhibition field occurred next to the youngest primordium on the apex. In this case, instead of attaining a whorled arrangement following the transition to flowering, an initial pseudo-whorl of primordia may have been perpetuated, successive minima appearing alongside only weakly inhibitory existing primordia.

At the core of the hypothesis advanced is the idea



that an increased number of primordia on the apical surface can lead to a more uniform inhibitory field around the perimeter of the bare apex, and thence to a change to whorled or pseudo-whorled phyllotaxis. The change from whorled to spiral phyllotaxis that eventually occurred on reversion (Section 4.2.2, p. 80), may involve a slightly different mechanism, since the problem then is to induce asymmetry in the inhibitory field. This question is dealt with in a later section (p. 253). The next problem is to explain how the increase in number of primordia on the apical surface that occurred on the transition to whorled or pseudo-whorled phyllotaxis may have been brought about.

The importance of changes in the ratio of primordium to frustum size at initiation in determining changes in the number of primordia on the apical surface

An increase in the number of primordia on the apical surface, from three in the vegetative (LD) apex to about eight in the flowering and reverting apices (Section 4.2.3, p. 86) would seem to imply that, for a time, primordia were initiated more rapidly than frusta on transfer to SD. This could occur if the size of the primordium at initiation decreased before the length of the frustum at initiation, if the following two assumptions are made: firstly, that the growth rate of the apex was the same in the radial and vertical directions; secondly, that frustum size at initiation was proportional to primordium size at initiation. The following sequence of events might then occur on transfer to SD. Initially there are three primordia on the



apical surface. After transfer to SD the apex begins to initiate primordia that are smaller at initiation than previously. Meanwhile, the three primordia already present, of larger size at initiation than the primordia within, move to the outer perimeter of the apical surface (the area of which remains constant during the transition - see Section 4, p.86 ), and a frustum is initiated with each of them. Because frustum size at initiation is proportional to primordium size at initiation, the three frusta initiated will be the same size at initiation as during vegetative growth, even though the apex has started to initiate smaller primordia during the time these outer primordia leave the apical surface. When the first of the smaller primordia initiated by the apex reaches the outer perimeter of the apical surface, frustum size at initiation will decrease. There is therefore a period of time when the apex initiates primordia that are smaller at initiation than in vegetative (LD) plants, whilst the frusta it initiates are the same size as those in vegetative (LD) plants. This means that the number of primordia on the apical surface will increase, because more growth is required to initiate a frustum than a primordium. The extent of the delay between the time at which primordium size at initiation decreases and the time at which frustum size at initiation decreases will be a function of the extent to which primordium size at initiation decreases. This will therefore determine the extent to which the number of primordia on the apical surface increases.

Some indication that such a mechanism operated during



the transition from spiral to whorled or pseudo-whorled phyllotaxis was obtained, as is illustrated in Figure 50. The ratio between the area of the primordium at initiation (relative to the area of the apical surface) and the length of the frustum at initiation (relative to the length of the tissue generating the frustum) varied between about 1.0 and 1.7 in vegetative (LD) plants sampled at different stages during the experiments. During the Mid petal stage in flowering plants, and the Intermediate part/leaf stage in reverting plants, the ratio fell to between about 0.5 and 0.9. This was because the area of the primordium at initiation had decreased more, by this stage, than the length of the frustum at initiation. This suggests that the increase in the number of primordia on the apical surface, from three in vegetative plants to between seven and 10 in flowering and reverting plants (see Table 15, p. 87), may have resulted from a difference in the rate at which the sizes of the primordium and the frustum at initiation decreased following transfer of the plants to SD. The stem frustum appears to have decreased in size at initiation when the smaller primordia reached the perimeter of the apical surface, suggesting that frustum size at initiation may have been proportional to primordium size at initiation, as was assumed earlier (p.247). This may have been because the size of the frustum at initiation was determined by the size of the primordium when it was initiated. Thus the frustum could be determined before it was initiated, just as the primordium itself is determined before it is initiated (Snow and Snow, 1933).



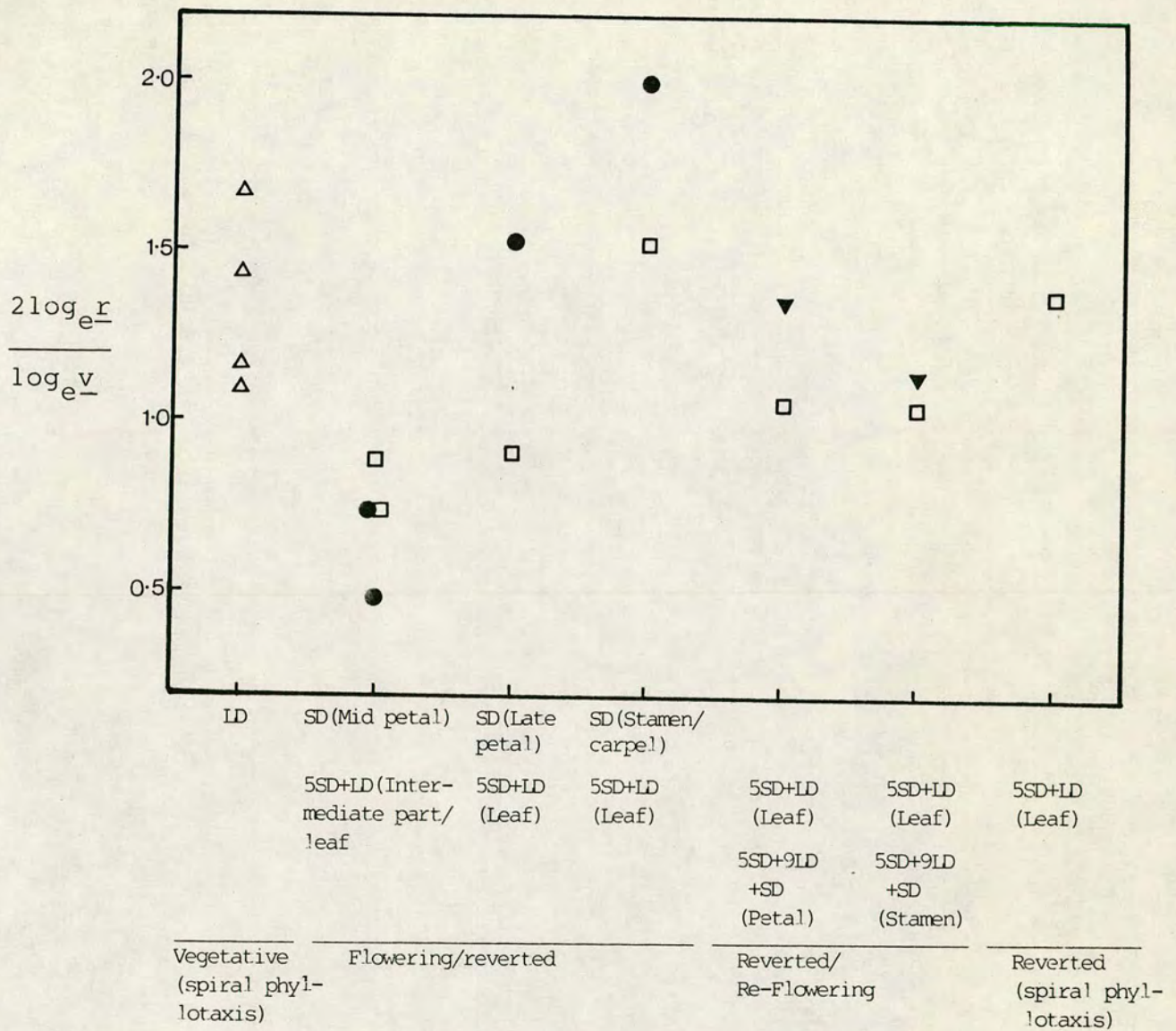


Figure 50. Ratio of the area of the primordium at initiation (relative to the area of the apical surface) to the length of the frustum at initiation (relative to the length of the tissue generating the frustum) ( $2\log_e r / \log_e v$ ) at different stages of development of plants treated with LD (Δ), SD (●), 5SD+LD (□), and 5SD+9LD+SD (▼).



At the Stamen/carpel stage in flowering plants, and the equivalent Leaf stage in reverted plants, the ratio between the sizes of the primordium and the frustum at initiation increased to between 1.5 and 2.0 (Fig. 50). It was at this stage that the number of primordia on the apical surface declined to about five or six in both flowering and reverted plants (see Table 16, p.93 ). This suggests that an increase in the area of the primordium at initiation relative to the length of the frustum at initiation may have resulted in a decrease in the number of primordia on the apical surface. However, the area of the apical surface also decreased slightly at this stage (Table 16, p.93 ), and this may also have played a role, since primordia would leave the apical surface sooner than at the previous stage. The data for this stage also call into question the idea that frustum size at initiation is proportional to primordium size at initiation, since the area of the primordium increased only slightly (Table 16, p.93 ), whilst the length of the frustum decreased and did not increase subsequently (Table 19, p.108). This suggests that, in at least some cases, the sizes of the primordium and the frustum at initiation are under separate control. The assumption that the area and vertical growth rates of the apex did not change relative to one another, seems to be reasonable, since apical shape appeared to remain roughly the same during vegetative growth, flowering, and reversion (see Figure 15, p.89 ). The actual measurements of apical growth rate obtained for Impatiens were derived



as the product of the size of the primordium or frustum at initiation and the rate of primordium initiation. They therefore have the disadvantage that it is assumed that the rate of primordium initiation is the same as the rate of frustum initiation. As we have seen, when the number of primordia on the apical surface is changing, this is probably not true. Therefore estimates of vertical relative growth rates may be subject to some inaccuracy if derived in this way when the number of primordia on the apical surface is not constant. Direct measurements of the rates of apical growth in the radial and vertical dimensions during the times at which the number of primordia on the apical surface changes would be desirable, since a constant ratio of primordium to frustum size at initiation could be associated with an increase in the number of primordia on the apical surface, if the area relative growth rate increased relative to the vertical relative growth rate. This might provide an alternative, or complementary, mechanism, to that proposed here.

In Impatiens, then, changes in the ratio of primordium to frustum size at initiation appear to result in changes in the number of primordia on the apical surface, and so may indirectly influence primordium arrangement at the apex (see p.244). Could such a mechanism operate in other species during changes in primordium arrangement? In species which have a domed apex, changes in the ratio of primordium to frustum size at initiation might be expected to result in changes in the



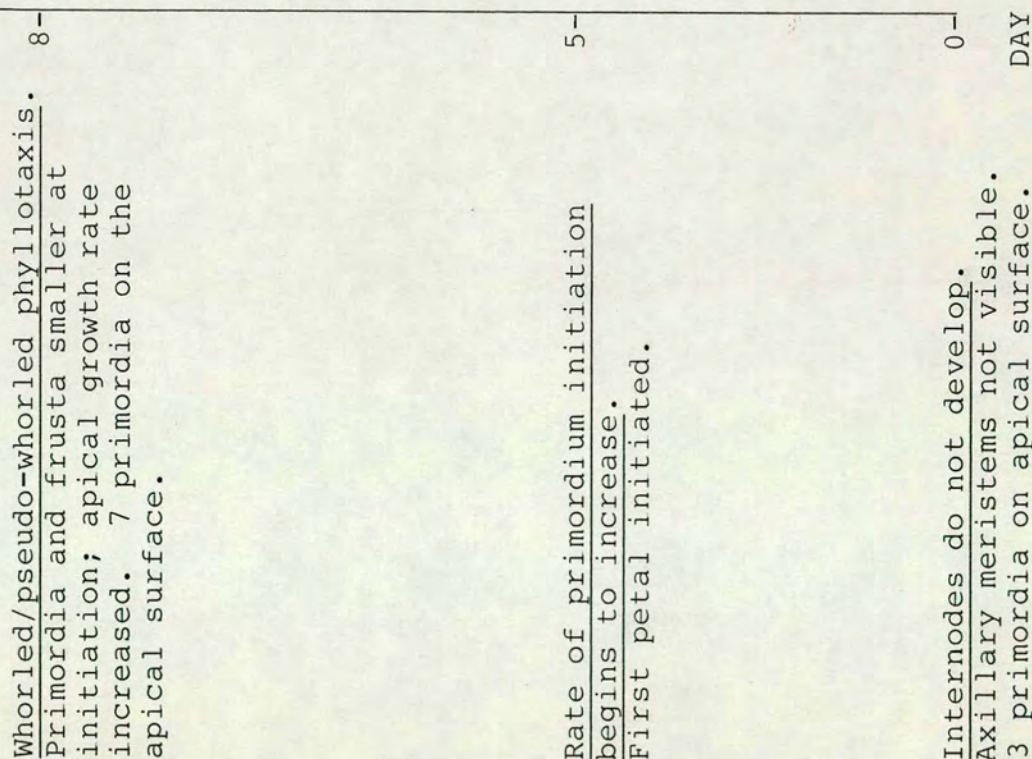
number of primordia immediately below the apical surface. This is what occurs in Epilobium adenocaulon, E. vitellinum (Schwabe, 1984b), and E. hirsutum (Meicenheimer, 1982), in which the vertical spacing between the primordia is less in flowering apices than in vegetative apices. However, since apical shape also changes on flowering in these species, changes in the ratio of area to vertical relative growth rates may also be involved. Also, in these species phyllotaxis changes from decussate to spiral on flowering, so that the number of primordia at a node decreases from two to one. Thus, in this sense, the problem is to explain how the initial asymmetry is induced so that instead of arising simultaneously in a two-membered whorl, primordia arise singly with a divergence angle of  $137^{\circ}$ . This is similar to the change that occurs when reverted Impatiens plants change from whorled to spiral phyllotaxis, and is discussed in detail in the next section.

The sequence of changes on flowering and reversion;  
a possible role for internode growth in controlling  
phyllotaxis?

On reversion, the measured growth parameters did not all return to their original vegetative (LD) levels at the same time. The sequence in which they were reversed is summarized in Figure 51. The sequence of changes on flowering is included for comparison. It is striking that internode development was affected very early on both on flowering and on reversion. Although



FLOWERING IN SD



REVERTING IN LD AFTER 5SD

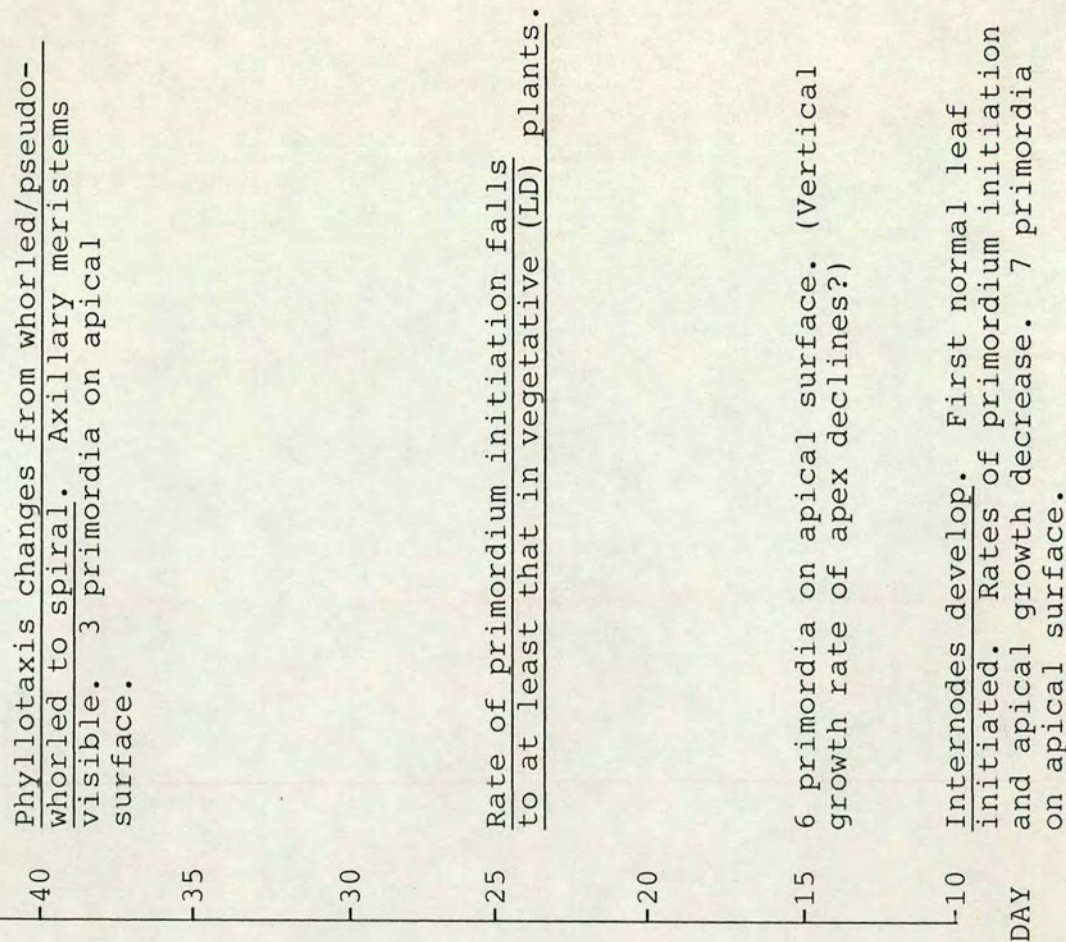


Figure 51. Summary diagram illustrating the sequence of events in plants flowering in SD and reverting in 5SD+LD.



there was a slight decrease in the rate of primordium initiation and apical growth at about the same time as internodes returned on reversion, dramatic changes in the rates of primordium initiation and apical growth appeared to follow the changes in internode development both on flowering and on reversion. This was measured as a marked decline in the rate of primordium initiation between days 25 and 42 in reverted plants (Section 4.2.1, p. 74 ). Although no direct measurements of apical growth are available for this period, an estimate can be made. If the minimum sizes of primordia and frusta at initiation (relative to the size of the generating tissue) are taken as those measured in reverted plants at the previous developmental stage (the Leaf (34-49) stage - see Tables 16 and 19, p. 93 and 108), and the maximum sizes taken as those measured in reverted plants after the return to spiral phyllotaxis (see Tables 17 and 20, p. 95 and 111), then the area relative growth rate per day would be between 0.12 and 0.22, and the vertical relative growth rate per day between 0.08 and 0.16. Since the area and vertical relative growth rates per day in reverted plants sampled before day 25 (at the Leaf (34-49) stage) were 0.51 and 0.33 respectively (Table 22, p. 114), the implication is that the early decline in apical growth rates measured on resumption of leaf initiation (on about day 10) was markedly accentuated between days 25 and 42. It is reasonable to conclude that



the change from whorled or pseudo-whorled to spiral phyllotaxis on about day 40 was preceded by a marked decrease in both the rate of apical growth and the rate of primordium initiation.

On reversion, then, internode development resumed; the rates of apical growth and primordium initiation probably decreased markedly; and finally the phyllotaxis changed from whorled or pseudo-whorled to spiral. On flowering, although internode development was inhibited on transfer to SD (internodes were less than 1 mm long above part 8.4 (1 s.e.=0.2, n=77)), it is not clear whether the increase in the rate of apical growth preceded, or accompanied, the change from spiral to whorled or pseudo-whorled phyllotaxis. In the case of reversion, it is reasonable to ask whether renewed internodal growth below the apex might have caused the marked decrease in the rates of primordium initiation and apical growth between days 25 and 42. This might have resulted from diversion of assimilates to the internode tissues, so that the apical meristem was deprived of materials required for maintenance of rapid growth and primordium initiation. Conversely, during the transition to flowering, inhibition of internode growth could have resulted in the more rapid rates of apical growth and primordium initiation that occurred during the transition to flowering, by causing increased availability of nutrients to the apical meristem. Thus, an early effect of photoperiod on internode growth would indirectly cause changes in apical growth. This is essentially the idea behind



the 'nutrient diversion hypothesis', in which the effects of chemicals on flowering are explained in terms of modifications of sink strength of competing vegetative tissues or of the apical meristem itself (Sachs and Hackett, 1977). Whilst this hypothesis has many attractions, in that it offers a single explanation for the effects of very varied treatments on the growth and development of the shoot apex, it does not state in specific terms how the complex changes associated with flowering result from increased assimilate supply. This problem is also apparent here. For, at the transition to flowering, increased assimilate supply to the apical meristem might allow increased rates of apical growth and primordium initiation, but it is difficult to see how it could cause the change in phyllotaxis from spiral to whorled or pseudo-whorled. However, inhibition of apical growth after reversion might influence the change from whorled or pseudo-whorled to spiral phyllotaxis as follows.

It was a common observation that members of the whorls or pseudo-whorls of leaves on the reverted meristem differed slightly in size and/or level of insertion on the stem (see, e.g., Fig. 24B). This might have resulted from slight differences in the size at initiation and time of initiation of the members of each whorl or pseudo-whorl (see also p. 236). If the apex was growing relatively slowly, then it might only generate sufficient cells to initiate one primordium at a time. Such asymmetries as already existed in the level of insertion, and size, of the members of a whorl/ or pseudowhorl, would then



be progressively enhanced. It was noted in dissection of mature reverted plants that the return to spiral phyllotaxis occurred gradually, since it was almost always preceded by a phase in which primordia were initiated in a very irregular fashion, sometimes in threes, twos, and sometimes singly. Other studies suggest that a slight difference in vertical spacing of the members of a whorl may result in a change to spiral phyllotaxis. In Epilobium hirsutum a change in the relative vertical spacing of one of a pair of primordia was associated with the change to spiral phyllotaxis (Snow and Snow, 1937). Auxin application instigated asymmetry in the bijugate system by causing one of the pair of  $I_1$  primordia to fuse with the  $P_1$  below it, so that it was kept down to a lower level on the apex than its  $I_1$  partner (see Fig. 14, Snow and Snow, 1937). Meicenheimer (1981), working on the same species, found that application of an inhibitor of auxin transport to one of a pair of primordia resulted in fasciation between the treated primordium and one of the next primordia to be initiated. When this resulted in a change in the relative vertical spacing of the primordia, a change from bijugate to spiral phyllotaxis also occurred. In Dryopteris dilatata, bijugate apices frequently switched to spiral phyllotaxis following removal of the older primordia on the apex to allow observations and measurements to be made (Cutter, 1964). In discussing this finding, Cutter states: "The observed change from bijugy to spiral phyllotaxis is usually a result of a pair of leaf



primordia becoming unequal in size and situated at slightly different levels." And also: "In Dryopteris, bijugy is probably only approximate; and existing slight differences in size and level of insertion between the two primordia of a pair could be enhanced by a lengthening of the plastochrone, which has been shown to occur over a period of time, and probably also be increased internodal growth, which seems also to occur under the experimental conditions." Although in bijugate apices only two primordia are initiated in each whorl, it seems possible that a similar mechanism may operate in/<sup>the</sup>switch from whorled or pseudo-whorled to spiral phyllotaxis in Impatiens.

I have suggested here that growth of the internodes below the apical meristem may, by depriving the apex of assimilates required for growth, lead to a lengthened plastochron and an enhancement of existing asymmetries in the whorled or pseudo-whorled phyllotaxis. Further work on Impatiens is needed to test the idea that, after reversion to leaf initiation, renewed internodal growth diverts assimilates from the apex. If internodal growth could be prevented on reversion, we might expect to prevent the subsequent change in phyllotaxis, if the hypothesis advanced is correct. There is some very limited evidence on this question from my own studies. In some early experiments, designed primarily to increase the uniformity of the reversion response, continuous light,



rather than LD, was used to bring about reversion after 5-8 SD (see Table 8, p.64 ). Although the uniformity of reversion was not improved, elongation of the internodes in reverted plants was very limited under these conditions. Furthermore, although dissections were carried out on about day 42, when the apices might only just be expected to have returned to spiral phyllotaxis, there was no sign of such a change in R4 reversion types, and in some cases the plants appeared to have returned to petal formation (see Table 8, p.64 ). This suggests that inhibition of internode development/prevented a return to spiral phyllotaxis; it is interesting that under these conditions the reverted plants may have begun to flower again. However, if this possibility were examined in more detail, it would be desirable to find a treatment that prevented internode development without giving the plants continuous light, since this in itself might affect the assimilate balance within the plants.

C. Summary of possible mechanisms involved in the changes in phyllotaxis on flowering and reversion; unanswered questions

The points made in this section can be summarized as follows.

- 1) During the transition to whorled or pseudo-whorled phyllotaxis the divergence angle may decrease from  $137^{\circ}$  to about  $36^{\circ}$ - $70^{\circ}$ . Whorled phyllotaxis seems to be explicable in the same terms as spiral phyllotaxis,



i.e. that young primordia produce an inhibitor of primordium initiation. The problem is to explain how the change from the initiation of one primordium, to the initiation of several primordia, at a node, is brought about.

- 2) An increase in the number of primordia on the apical surface may result in a more/<sup>or</sup>less symmetrical field of inhibition around the bare apex, which may in turn lead to a whorled or pseudo-whorled arrangement of primordia in flowering and reverting apices.
- 3) The increase in number of primordia on the apical surface may result from a transient decrease in the ratio of primordium to stem frustum size at initiation.
- 4) Internodal growth was affected very early on, on both flowering and reversion. It seems possible that renewed internodal growth below the reverted apex may lead to a decrease in apical growth rate, and that this may accentuate existing asymmetries in the whorled or pseudo-whorled phyllotaxis, eventually leading to a return to spiral phyllotaxis.

If these ideas are correct, then we need to know what controls primordium and stem frustum size at initiation. Also, further work is needed, particularly during the time that the reverted apex returns to spiral phyllotaxis, to determine the exact changes in primordium



arrangement that take place.

#### 7.1.4 The control of final internode length

In the preceding section we have seen that the changes in primordium arrangement on flowering and reversion are intimately related to changes in the sizes of primordia and stem frusta at initiation. We can also ask how the differences in the mature appearance of flowers and reverted flowers are related to these changes. A typical feature of flowers is an absence of internodes. Impatiens flowers were typical in this respect, and in R4 reversions internodes returned at the time that normal leaf production resumed (Table 11, p. 72). The absence of internodes in the flower might have occurred either because the internodes were initiated but did not develop, or because on initiation the stem frustum (the future node plus internode) was much smaller than in the vegetative plant. This question does not seem to have been considered before, although the absolute size of stem frusta during vegetative growth has been measured (Sunderland and Brown, 1956; Berg and Cutter, 1969; Smith and Rogan, 1975).

For about 15 days after resumption of leaf initiation in reverted (5SD + LD) plants the length of the stem frustum at initiation remained similar to that in flowering (SD) plants (Section 4.2.4, p. 94). This indicates that the length of the stem frustum at initiation did not determine the length of the stem frustum at maturity, since internodes developed in reverted plants, but not in flowering plants (Table 33, p. 159). However, the reduction in the length of the stem frustum at initiation may have been necessary for



the reduction in stem frustum length at maturity in the flower.

The question can therefore be asked: how did the large internodes (and therefore frusta) of reverted plants arise from frusta that were the same length at initiation as those in flowering apices in which internodes did not develop? This could have resulted from differences in the growth of the tissues of the sub-apical meristem in flowering and reverted plants. Factors operating after initiation, such as photoperiod and concentrations of growth substances, are known to alter activity of the sub-apical, rather than the apical meristem (Sachs, 1965). The data available for Impatiens are measurements of vertical growth of the tissues greater than a distance equivalent to 111.2  $\mu\text{m}$  below the apical surface (Section 4.2.4B, p.102). These tissues had about the same vertical relative growth rate per day in vegetative (LD), flowering and reverted plants (Table 22, p.114). Only the vertical growth rate per plastochron of these tissues was correlated with final frustum length in the different treatments (Fig. 18, p.103; Table 23, p.116). However, the difference between final frustum length in the mature tissues of flowering and reverted plants was in this case attributable to differences in the length of the nodal tissue, since internodes were not present between the intermediate parts of reverted plants, which were the only parts more than a distance equivalent to 111.2  $\mu\text{m}$  below the apical surface at the time the plants were sampled (Section 4.2.4B, p.102).



Therefore, further measurements need to be made on reverted plants at a later stage of development than those measured here, when the whorls or pseudo-whorls of leaves, initiated after the intermediate parts of the reversion zone, and separated by internodes at maturity, would have been displaced more than a distance equivalent to 111.2  $\mu\text{m}$  below the apical surface. Then it could be determined if increased growth in the tissues of the sub-apical meristem (relative to that in flowering (SD) plants) is responsible for the development of these internodes.

Final frustum length may also be controlled by the duration of frustum growth. Future investigation of this possibility would be of interest, since the final stages of cell elongation in the differentiating internode are known to be prevented by leaf removal, probably as a result of decreased auxin supply (Sachs, 1965). It may be, therefore, that one reason for the lack of internode development that accompanies flowering is the presence of floral parts (rather than leaves) which may not supply auxin needed for cell elongation in the internodes next to them.

## 7.2 Development of the primordia on flowering, reverting and re-flowering apices

### 7.2.1 The relationship between primordium development and apical development

The observations made on the types of organs produced by reverted and re-flowered apices have a bearing on the idea that flowering consists of a 'relay system',



in which each successive organ type depends on the preceding organ type for its initiation (Heslop-Harrison, 1964). In the R5 reversion type normal bracts and some normal petals formed without subsequent formation of stamens and carpels, and in the R6 and R7 types petals formed, followed by stamens and then petals again (Table 6, p.60 ). Therefore the formation of one type of floral organ does not necessarily result in the formation of all the other floral organs in Impatiens. Nor is the succession of parts irreversible in this species. Also, on return to SD reverted plants did not initiate bracts, but began petal initiation immediately (Section 5.1, p.125). The bracts initiated at the time these plants were first transferred to SD on day 0 were therefore followed by intermediate parts and, in some treatments, leaves, which were followed directly by petals. This means that if a relay system exists it can be disturbed without preventing

further organogenesis at the apex. However, in none of the experiments were stamens initiated without any preceding normal petals, suggesting that the apex must pass through a phase of petal initiation before beginning to initiate stamens. Similarly, although bracts were not initiated on re-transfer of reverted plants to SD, this may have been because these plants had, on first transfer to SD, already initiated bracts. Therefore, whilst the relay system can be temporarily suspended (as in reverted plants before re-transfer to SD), reversed (as in the R6 and R7 reversion types), or halted completely (as in reverted plants left in LD), there is no evidence that in



Impatiens it can be short-circuited as it can be in Silene coeli-rosa (Lyndon, 1979b). The fact that the postulated relay system can be temporarily suspended may mean that any factors produced by the preceding organ type (bracts in this case) that are necessary for the formation of the subsequent organ type (petals) are relatively long lived in the apex. This seems more likely than the possibility that bracts continue to produce the required factors for a considerable period of their development, in view of their mature leafy appearance in reverted plants (see Fig. 9, p.70 ) and their switch towards a more leaf-like shape when about 1.5 mm long (Section 6.1, p.166). On the other hand, if formation of petals in Impatiens occurs as a consequence of the arrival of a second stimulus from the leaves (leaves of reverted plants being partially induced - see p.232), then it seems unnecessary to postulate a relay system at all in this species. Resolution of these questions depends on the outcome of further experiments, designed (as described on p.283) to determine if reversion results from a requirement for more than one inductive stimulus from the leaves, or from a continued requirement for a single stimulus.

#### 7.2.2 Primordium commitment and its control

A. Commitment of primordia in Impatiens is unusual because it does not occur at the level of the whole organ, and is very late

On return to SD reverted plants not only began to initiate petals immediately, but there was also a



retrospective effect of SD on primordia already present at the time of re-transfer (Section 5.1, p.125). In plants given 5SD+9LD+SD, 13 primordia that would have developed as intermediate parts or leaves in reverted plants, developed as petals as a result of SD. This indicated that commitment of these primordia must have occurred after their initiation. Although the exact time that SD began to exert their effect at the apex is unknown, if it is assumed that SD began to affect primordium development immediately, then the beginning of SD fixes the earliest time at which commitment of any part of the primordium could take place, and provides a starting point for further work. Making this assumption, transfer of reverted plants back to SD after varying numbers of LD showed that primordia became committed to leaf development over a period of about 11 days, as they grew from 0 to about 750  $\mu\text{m}$  in length (Section 6.2, p.194). This gradual commitment occurred because the primordia did not become committed as a whole; the tip of the primordium generally became committed to leaf development before the base (see Fig. 40, p.199).

There is no information about the time of commitment to leaf (rather than petal) development in other species. This is because, just as flower reversions are unusual (because most species become committed to flower), so interconversions between leaves and petals are not usually observed. This suggests that the ability of the flower meristem to revert to vegetative growth in Impatiens may be correlated with the ability of the primordia



to switch from petal to leaf development on reversion, as they probably do when intermediate parts are formed (Section 6.1, p.166), and from leaf to petal development on re-flowering of the reverted meristem (Section 6.2, p.194). In preliminary experiments with Anagallis arvensis, flower reversions were also often accompanied by the formation of parts intermediate between petals and leaves. It seems that in both Impatiens and Anagallis an absence of commitment to flower is associated with very late commitment in the primordia initiated by the reverting apex.

In other species, various aspects of primordium commitment have been studied. In these cases a general distinction can be made between commitment of the primordium to be a particular organ type, and commitment of the form of the organ type. Isolation of potential leaf primordia on the apex of Dryopteris aristata (=D. dilatata) showed that these primordia could develop as buds until they were about two to three plastochrons old (Cutter, 1954). After this time they became committed as leaf primordia. Snow and Snow (1933) found that a primordium of Lupinus albus could be made to develop as a bract rather than a leaf if incisions were made in the apex up to half a plastochron before primordium initiation. After this time both the position and nature of the primordia were determined. Both these examples show that commitment of the primordium to be a particular organ type usually occurs very early in primordium development.

Commitment (or determination) of the form of the leaf



appears to occur somewhat later. For example, the degree of leaf dissection is not determined at primordium initiation in the aquatic heterophyllous species Ranunculus flabellaris. This is shown by the fact that intermediate leaf forms develop from primordia present at the time of transfer of plants from the aquatic to the aerial environment (Bostrack and Millington, 1962). In the aquatic plant Callitriche intermedia linear leaves are produced whilst the crown is submerged, and ovate leaves whilst it is above water. Intermediate forms between these two extreme types are found, which result from a change in the course of primordium development in response to submergence or arrival of the crown at the surface. The tip of the leaf became unresponsive to changes in environment before the base, and this was interpreted as being a result of the basipetal development of the leaf (Jones, 1956). Thus the form of the leaves of aquatic heterophyllous species is not determined at initiation, although it is not clear for how long the primordia remain uncommitted. In the case of Pisum sativum, the leaf primordium is able to regenerate a new leaf if damaged when very small, but its form is determined by the time it is 70  $\mu\text{m}$  long (Sachs, 1969).

The only data on the commitment of petal primordia are those of McHughen (1977), who concluded that the petal of Nicotiana tabacum was determined by the time it was initiated. This conclusion was based on the fact that fairly normal petal development occurred when the primordia were removed from the apex and cultured. However, no



indication was given of the smallest size of the primordia on transfer to culture, so there may have been a period when the primordium was very small when it was not determined. Also the ability to continue development when removed from the apex represents only one rather limited aspect of organ determination or commitment.

In the case of Impatiens commitment of the primordia does not seem to occur at the level of the whole organ, since some areas of the same organ can have petal characteristics whilst other areas are leafy, at maturity (see Figs. 8C, 9, 22, 40, p. 59, 70, / <sup>131 and 199</sup> ). Both the form of the organ, and the characteristics of the cells that make it up (e.g. the presence of petal pigment in the epidermal cells), remain undetermined for an unusually long time after initiation. The only comparable report is that of 'labile' primordia in Petkus rye, in which transfer of plants from short / <sup>days to long days</sup> well after initiation of these primordia caused them to grow into spikelets rather than leaves (Purvis and Gregory, 1937). The same is true in Chinese Spring Wheat (Griffiths, 1981). However, in these cases the primordia did not switch from one developmental pathway to another, since the primordium was a 'double ridge' structure. The balance between the growth of the outer and the inner parts of this primordium determined whether a spikelet or a leaf was seen at maturity.

B. What does commitment in Impatiens primordia involve?

The fact that the tips of the primordia on the



reverted apex of Impatiens usually became committed to leaf development before the base (resulting in a basipetal pattern of commitment to leaf development) suggests that in this case commitment may be associated with maturation of the primordium. In Nicotiana tabacum (Avery, 1933) and Linum usitatissimum (Girolami, 1954) leaf primordium maturation takes place basipetally. During leaf development cell divisions cease first at the tip of the leaf, and last at the base, and similarly cells at the tip of the leaf become differentiated earlier than those at the base (Dale and Milthorpe, 1983). Although the basipetal pattern of maturation becomes evident at different times in different species - apical growth continues in the tobacco leaf primordium until it is 2-3 mm long (Avery, 1933), whereas it ceases when the primordium is only 150  $\mu$ m long in flax (Girolami, 1954) - it may actually be established very early on in primordium development. This is suggested by the fact that, in Pisum sativum, the epidermal cells at the tip begin to divide more slowly at the moment of primordium initiation (Lyndon, 1982).

To investigate the possible nature of commitment, and whether it could be correlated with differences in cell differentiation times (as reflected by the time of vacuolation) in the tip and the base of the primordium, it was asked whether commitment to leaf development occurred before, at the same time as, or after vacuolation of the mesophyll cells at the tip and the base of the primordium. There is evidence that sensitivity of cells of primordia of Spirodela polyrrhiza to abscisic acid (ABA)



disappeared with the onset of rapid cell expansion (Smart and Trewavas, 1983). Primordia were sensitive to ABA, developing as turions in its presence, and vegetative fronds in its absence, only when they were less than about 700  $\mu\text{m}$  long. After this time rapid cell expansion began and the primordia became progressively less sensitive to ABA. There are other interesting parallels between Spirodela and Impatiens. In both species primordia intermediate between two extreme forms were produced. In Spirodela, semi-turions resulted from ABA treatment of fronds between 700 and 1300  $\mu\text{m}$  long; semi-turions had a turion-like proximal end and a frond-like distal end. In Impatiens intermediate parts resulted from re-transfer of reverted plants back to SD when the primordia were between about 100 and 700  $\mu\text{m}$  long (Fig. 39, p.198). These had a petal-like proximal end and a leaf-like distal end (Fig. 40, p.199). In both species the proximal end of the primordium was the last to be committed - to leaf development in Impatiens, and to vegetative frond development in Spirodela. It is interesting that in Figure 3(a) of Smart and Trewavas (1983) the distal end of a primordium 700  $\mu\text{m}$  appears to have been much more vacuolated than the proximal end. This supports the idea that in Spirodela there may be a correlation between the ability of the different parts of the frond to respond to ABA (and develop as a turion) and the absence of substantial vacuolation.

In Impatiens the proportion of the cell occupied by vacuole began to increase after primordium initiation, and



increased steadily during the time primordia became committed to leaf development (Section 6.2, p.194). The absolute volume of vacuole per cell increased during this time, associated with an increase in cell volume. Thus, it is clear that there is a correlation between cell vacuolation and commitment to leaf development. However, there were no marked differences between the four areas of the primordium selected for analysis. The early commitment of the tip, and the later commitment of the base of the primordium, was not reflected in an earlier onset of vacuolation of the tip compared with the base. This suggests that although vacuolation may be generally involved with commitment to leaf development, other factors must also play a role. It would be of interest to measure the extent of vacuolation in the cells of the primordia on the vegetative (LD) apex at the time of transfer to SD on day 29. Commitment is apparently much earlier in these primordia than in those on the reverted apex (Section 5.1, p.125). If vacuolation is involved with the commitment process, then it should occur much earlier in the primordia on the vegetative (LD) apex than in those on the reverted apex. However, it would be necessary to establish first of all that this apparent difference in commitment time was not a reflection of a difference in the stage of primordium development at which the SD stimulus reached the primordia on the vegetative and reverted apices.

The detailed stereological analysis (Section 6.2, p.194), showed that plastid numbers per cell and plastid volume remained roughly constant during the phase of



primordium development studied. About 50-60 plastids were present in each cell. This was about the number found in spinach leaf cells at the earliest stage of leaf development (leaf length of about 2 cm) measured by Possingham and Saurer (1969), and in the region immediately above the intercalary meristem of wheat leaves sampled by Boffey, Ellis, Sellden and Leech (1979). The constant number of plastids per cell during commitment in Impatiens implies that plastid division must have roughly kept pace with cell division at this stage of primordium development. Later in leaf primordium development an increase in the number of plastids per cell would probably take place, as in spinach (a six-fold increase) and wheat (a three-fold increase) (Possingham and Saurer, 1969; Boffey et al, 1979). This would be associated with the attainment of the full photosynthetic capacity of the leaf (Leech and Baker, 1983). It would be of interest to know whether such an increase takes place if Impatiens primordia are switched into petal development by transfer to SD before primordium commitment. If not, then commitment would obviously exert an influence over plastid development. Changes in the number of plastids per cell or plastid volume are clearly not involved with the process of commitment to leaf development itself.

A possible mechanism producing the basipetal pattern of commitment in the primordia on the reverted apex is suggested by the similarities between intermediate parts in Impatiens and petals infected by mycoplasmas in Vinca rosea. Intermediate parts on the reverted or



re-flowered apex typically had areas in which the epidermis contained petal pigment (a characteristic of petals), whilst the mesophyll cells had chloroplasts (a characteristic of leaves) (Figs. 28 and 29, p. <sup>167</sup>/<sub>170</sub> & ). In green petals that result from infection of Vinca by mycoplasmas, the cells of the petal mesophyll contain chloroplasts which are typically absent in normal petals (Gourret, 1971). The effect of mycoplasma infection is particularly noticeable around the phloem in these petals, and this is interpreted as being a result of the mycoplasmas setting up a new balance of plant growth regulators around the phloem in which they reside (Gourret, 1971). The effect is thought to stem from a very early change in the development of these petals, which diverts the cells into the developmental pathway of leaf cells. If the signal(s) causing cells to develop as petal cells are also carried in the phloem in Impatiens, then different rates of phloem maturation in different areas of the primordium might cause these areas to develop differently. Those areas which became committed to leaf development earliest would have been those in which phloem matured earliest, before arrival of the SD stimulus. This would imply that, following phloem maturation, the commitment step occurs in the cells of the primordium. If the SD stimulus is not present in the materials supplied by the phloem, then commitment would be to leaf development. If it were present, then the cells would develop as petal cells. It is not clear, however, why the commitment step might be taken following phloem maturation; neither is the juxtaposition of epidermal cells with petal pigment and mesophyll cells



with chloroplasts (see Figs. 28 and 29, p.<sup>167 &</sup>170 ) accounted for. Although vascular tissue could be seen to be present in the transmission electron micrographs used for the stereological analysis (Section 6.2, p.194), more photographs, preferably of transverse sections of the primordia (to allow easy identification of phloem), would be needed to determine whether phloem maturation proceeds basipetally, and whether it parallels the process of commitment to leaf development.

We have seen that commitment to leaf development of the primordia on the reverted apex of Impatiens occurs relatively late during primordium development, and that it takes place earlier in the tip of the primordium than in the base. However, the mechanism of commitment remains obscure. It appears that vacuolation occurs at about the same time as commitment, suggesting that this aspect of cell differentiation may begin at about the same time as commitment. However, it is not clear how commitment is related to cell differentiation in the primordium - does it instigate differentiation, or is it caused by the onset of differentiation, so that cells can no longer alter their pathway of development because of irreversible cellular changes associated with differentiation? A possible solution to this problem is presented in the next section.

### 7.2.3 Commitment of the primordia and commitment of the apex

#### A. Two types of commitment revealed by Impatiens

Although commitment was very late in the primordia



on the reverted apex of Impatiens, it did eventually occur. On the other hand, the apical meristem does not become committed to flowering, even after formation of the placenta (Krishnamoorthy and Nanda, 1968). This suggests that these two types of commitment are different. Furthermore, in other species, commitment of a primordium to be a particular organ type appears to occur very early in primordium development (Snow and Snow, 1933; Cutter, 1954, 1965), at a time when the primordium consists of a group of relative<sup>ly</sup> uniform meristematic cells (Cutter, 1965; Esau, 1977). Commitment of the primordia on the reverted apex of Impatiens occurs at a time when the cells are vacuolating and beginning to differentiate; it is at this later stage that the form of the primordia in species such as Ranunculus flabellaris and Callitriche intermedia appears to become determined (see p.269). This suggests that, in Impatiens primordia on the reverted apex, the early commitment to be a particular organ type is absent, just as commitment of the meristem to flower is absent. The commitment that occurs later in the development of Impatiens primordia may result from the cells having begun to differentiate as leaf cells, so that they can no longer respond to the SD stimulus. If this interpretation is correct, then it suggests that in other species commitment of the meristem to flower may also result in the initiation of floral organs which are committed to be a particular organ type at about the time they are initiated.



B. What is the nature of commitment of the apex to flower?

In Impatiens, an absence of commitment to flower seems to result in a continued dependance of the apex on SD (flowering) stimulus during flowering itself. In species in which commitment to flower does occur, commitment may, therefore, result in the apex becoming autonomous for production of floral stimulus. If this were so, then after commitment the apex should no longer be dependent on the leaves for supply of floral stimulus.

In general there is little direct evidence as to whether the apex requires the floral stimulus itself after commitment. Certainly, many species, including Pharbitis nil, Xanthium pennsylvanicum (= X. strumarium) and Lolium temulentum (which initiate flowers in response to a single inductive cycle), show more rapid flower development if maintained in continuous inductive conditions (Eguchi, 1937; Hamner and Bonner, 1938; Naylor, 1941; Van de Pol, 1972; Evans, 1960; see Vince-Prue, 1975 for review). In Chrysanthemum morifolium floret initiation and inflorescence development is dependent on short days (Schwabe, 1951); whilst in Caryopteris x clandonensis flower bud initiation, and in Bougainvillea inflorescence initiation, are daylength indifferent but short days are required for complete flower or inflorescence development (Piringer, Downs and Borthwick, 1963; Hackett and Sachs, 1968). Do these effects of daylength on flower or inflorescence development indicate a continued requirement for floral stimulus during flowering? In Bougainvillea it has been proposed that, <sup>in unfavourable</sup> conditions, the young leaves export gibberellins



to the shoot apical and sub-apical regions, thereby inhibiting inflorescence development (Sachs and Hackett, 1977), whilst in Chrysanthemum high auxin levels might have contributed to the inhibitory effect of long days (Schwabe, 1951). In Xanthium the rate of development of a flower bud after one short day was not altered by removal of all other buds (Salisbury, 1963). This could mean that the amount of floral stimulus was not the factor limiting the rate of flower bud development in long days. Alternatively, it could mean that the rate of flower bud development was a function of the total amount of floral stimulus produced as a result of induction, rather than of the amount available to each bud (Vince-Prue, 1975). In Pharbitis, if the plants were grown at high temperature after a single inductive short day, reversion occurred at certain axillary meristems (see Introduction, p.12 ) (King and Evans, 1969). The authors concluded that the floral stimulus was thermolabile, and that there was a continued requirement for its action even after floral evocation. On the other hand, Zeevart (1962) concluded that the floral stimulus must be very short lived in Pharbitis, since flowering was prevented by 5-fluorodeoxyuridine application at the beginning of the long night, even though cell division in the treated apices resumed about 48 h after application of the inhibitor. He therefore estimated that, since it arrived at the apex about 24 h after the beginning of the long night, the floral stimulus only had a lifetime of about 24 h in the apex. If the floral stimulus only had a lifetime of about 24 h at the



apex of induced plants (not treated with 5-fluorodeoxyuridine), and there was a continued requirement for floral stimulus during flowering, then reversion or arrested flower development should have occurred in these plants, because flower part initiation does not begin until about 87 h after the beginning of the long night (King and Evans, 1969). However, no reversions occurred, and flowers were formed, suggesting that floral stimulus was only required during evocation. A similar interpretation can be made of experiments designed to measure the time at which floral stimulus arrives at the apex of Pharbitis, Xanthium, Lolium and Sinapis alba (Zeevaart, 1962; Salisbury, 1963; Evans and Wardlaw, 1966; Bernier, Bodson, Kinet, Jacqmard and Havelange, 1974). In each species removal of the induced leaf well before flower initiation (16-35 h after the start of induction) did not prevent flower initiation. However, no data are available on the lifetime of the floral stimulus in the apex of Xanthium, Lolium or Sinapis.

Therefore, it is not clear whether the floral stimulus itself is required after commitment and during flower initiation. Often, it appears that completion of flower initiation and development is dependent on growth regulators supplied by the leaves (Vince-Prue, 1975). The floral stimulus is thought not to be the same as any one of the known growth regulators (Zeevaart, 1976; Bernier et al, 1981b)

If, after commitment, the meristem is no longer dependent on the leaves for floral stimulus, but, by analogy



with Impatiens, formation of the flower does require floral stimulus, the implication is that the meristem may develop the capacity to synthesise floral stimulus on commitment. This may be similar to the situation in tobacco pith cultures, which can habituate (Gautheret, 1955) for synthesis of both auxin (Cheng, 1972; Syono and Furuya, 1974) and cytokinin (Meins and Binns, 1979; Meins, Lutz and Binns, 1980). There is evidence that the ability to habituate for synthesis of these growth regulators parallels the ability of isolated stem segments of tobacco to initiate flower buds (Tran Thanh Van, 1973; Turgeon, 1982). Furthermore, a phenomenon similar to habituation may occur in plants in which the apex and young leaves undergo indirect induction. This has been shown to occur in Xanthium strumarium (Zeevaart, 1958; Lona, 1959), Bryophyllum daigremontianum (Zeevaart and Lang, 1962; van de Pol, 1972), Silene armeria (Wellensiek, 1966), and Perilla ocymoides (green-leaved) (Zeevaart, 1984).

If habituation for synthesis of floral stimulus occurs on commitment of the apex to flower, then it may be that the primordia initiated during flowering are committed at the time of their initiation because the cells from which they develop are habituated for synthesis of floral stimulus. Impatiens may not be able to habituate, so that both the meristem and the primordia it initiates are not committed. The implication is that the floral stimulus acts in such a way as to canalize development (whether of the meristem or of the



primordia); the developmental pathway followed by the responding tissue would presumably be a function of the competence of the cells on which the floral stimulus acts. If the ability to habituate for synthesis of auxin or cytokinin reflects the ability to habituate for synthesis of floral stimulus, then cultured tissues of Impatiens might be expected not to habituate. This could be tested experimentally.

### 7.3 Areas for future work on flowering; the value of Impatiens in this work

#### 7.3.1 The component processes of induction and evocation

We have seen (Section 7.1.2, p.232) that the response of the reverted apex to further SD could reflect either the successive action of several components of the floral stimulus at different stages of flower development, or a modified response of the partially evoked apex to a single inductive stimulus. Future work should aim to distinguish between these two possibilities, because we need to know whether more than one stimulus controls flowering, not only in Impatiens, but also in other species. Alternatively, if partial evocation involves a semi-permanent change in the response of the meristem and the young primordia to the same inductive stimulus, we can ask whether the correlation between this change and the attainment of whorled or pseudo-whorled phyllotaxis reflects control of primordium sensitivity to SD by the arrangement of primordia at the apex. Such control by primordium positioning is difficult to understand, but might reflect



spatial interactions between meristematic cells and the SD stimulus. An alternative possibility is that the primordia on the reverted meristem are made up of cells that have altered competence to respond to SD stimulus. How stable might this change be? It might be that, on culture, these primordia could regenerate whole plants whose response to SD, and phyllotaxis, was more like that of reverted plants than vegetative (LD) plants. The molecular basis for such a change might lie in the ability of the cells to synthesise growth regulators, particularly auxin, which seems a likely candidate for the inhibitor of primordium initiation that may control phyllotaxis (Schwabe, 1984b). Alternatively, or additionally, new receptor sites for the SD stimulus might be synthesised by the cells of the primordia on the reverted apex.

An experiment was in fact designed to determine whether the increased reactivity of reverted plants to SD resulted from changes that occurred in the leaves (partial induction of the leaves in response to 5SD+LD) or the apex. Reverted scions (with all expanded leaves removed) would have been grafted on to vegetative (LD) stocks. The grafted plants would then have been transferred to SD and their flowering response analysed at maturity. The presence of bracts in the flower, and a very limited effect of SD on primordia already present at the time of transfer (the flowering response of vegetative (LD) plants on transfer to SD), would have implied that it is the processes taking place in the leaves that are modified by the 5SD+LD treatment. On the other hand, flowering of the



type usually obtained when reverted plants are returned to SD (Section 5.1, p.125) would have indicated that the sensitivity of the apex to SD is altered by the 5SD+LD treatment. However, the experiment could not be performed because of difficulties encountered in grafting Impatiens. The extremely succulent nature of the stem meant that it was very difficult to prevent desiccation of the grafts, and no successful graft unions were obtained in preliminary experiments. A possible alternative method would be approach grafting, which was not attempted. Other experiments which would not involve grafting could also be designed. For instance, if different leaves were given the first 5SD from those that received the second SD treatment, then, in the absence of indirect induction (which seems unlikely because Impatiens reverts in LD) partial induction could be distinguished from an increase in apex sensitivity to SD by an analysis, similar to that described above, of the mature form of the flower.

Until experiments such as these have been done, partial induction cannot be ruled out as a possible explanation of the increased reactivity of reverted plants to SD. It is also worth noting that whilst partial evocation could result from a requirement of the apex for inductive signals throughout flower formation (because evocation accompanies flowering rather than precedes it), it might also be caused by the apex becoming incompetent to respond to inductive signals in LD. This would imply that the leaves become permanently induced following 5SD treatment, but on return to LD the apex response to the stimulus is blocked. The plumule has been shown to be



responsive to light break treatments in Pharbitis nil (Gressel, Zilberstein, Porath and Arzee, 1980), although in Hordeum vulgare no response occurred when only the apex was given long days (Dale and Wilson, 1979). This possibility could be tested in Impatiens by covering the apex of plants given 5SD+LD, so as to give the leaves LD and the apex SD, or by illuminating only the apex with a fibre optic probe, while the rest of the plant received SD after a 5SD+9LD pre-treatment.

Research could also usefully be done into the factors controlling primordium and frustum size at initiation. It has been suggested here that the ratio of primordium to frustum size at initiation may be important in determining the changes in phyllotaxis that occur on flowering (p247). If this is so, then it might be expected that when the reverted apex changes from whorled or pseudo-whorled to spiral phyllotaxis, the ratio would increase. This possibility could be studied in detail during the transition between days 25 and 42 (see Section 7.1.3, p. 253).

#### 7.3.2 Commitment - a key event?

If commitment to flower does involve a process analagous to habituation (Section 7.2.3, p.278), then it might be possible to cause habituation for synthesis of floral stimulus in relatively large amounts of tissue in vitro. This, in turn, might give a better chance of isolation and identification of the active principle. Such identification still seems to be critical to allow a better



understanding of flowering. Therefore, in as much as it may be able to throw light on this, commitment may be a key event for future study.

On the other hand, we also need to understand how the floral stimulus causes changes in the form, and pattern of initiation, of the parts on the apex. Here commitment does not seem such a critical problem, because the mechanisms of changing phyllotaxis (see Section 7.1.3, p.235), and primordium development as a leaf or a petal, seem most approachable in plants such as Impatiens, where commitment is absent, and where, as a consequence, the direct response of the apex to inductive signals can be analysed. In the case of primordium development, further study should concentrate on the cellular and molecular changes in the primordia on the reverted apex on return to SD. The role of phloem maturation in determining the fate of the cells of these primordia might be particularly worthy of attention, in the light of the similarities between intermediate parts and petals infected by mycoplasmas (see p.274).



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APPENDIX 1



## APPENDIX 1.

plants showing inflorescence reversion

Species	Response Type	Conditions giving rise to reversion	Characteristics of reverted plants	Reference
<u>Beta vulgaris</u> L.	LDP	After vernalisation, exposure to continuous illumination, then to short days.	Reverted to vegetative state, or with about 30 days continuous illumination, formed an aerial rosette.	Margara, 1960
<u>Brassica oleracea gemmifera</u> L.	DNP	Plants transferred from cold conditions to warm during flower development.	Apical buds developed into a group of leafy shoots with "generative" leaves. Plants then bolted and continued vegetative growth.	Stokes and Verkerk, 1951
<u>Bryophyllum daigremontianum</u> L.	LSDP	Plants induced by gibberellic acid treatment, then transfer to short days. 2 months high temperature caused reversion.	Inflorescence reversion, with return to leaf initiation with vegetative axillary shoots.	van de Pol, 1972
<u>Cheiranthus cheiri</u> L.	DNP	Induction by low growth temperature ( $2-5^{\circ}\text{C}$ ); return to $22-24^{\circ}\text{C}$ at young inflorescence stage.	Return to initiation of leaves with vegetative branches; zone of leafy bracts and aborted flowers on transition.	Diomaiuto-Bonnand, 1970, 1972 1975, 1976



APPENDIX 1. Continued

Species	Response Type	Conditions giving rise to reversion	Characteristics of reverted plants	Reference
<u>Chenopodium rubrum</u> L.	SDP	2 short days given to 3 day old plants, followed by long days.	Initial inhibition of leaf growth and release of axillaries from apical dominance reversed, and bud growth inhibited again. Terminal flower primordia develop as leaves (, also a flower reversion).	Seidlová & Opatrna, 1978
<u>C. amaranticolor</u> L.	SDP	2 short days followed by long days.	Increased rate of primordium initiation associated with inflorescence formation, but primordia develop into leaves rather than bracts.	Thomas, 1961a and b.
<u>Chrysanthemum morifolium</u> : var 'Blanche Poitevine' var. 'Mayford Crimson'	SDP	15-20 short days (inductive); transfer to 12 hr days with night breaks. 15 short days followed by long days.	Green centre to the inflorescence in 100% of the plants. After initiation of involucre bracts no reversion occurred.	Parmentier and Kinet, 1980. Mitra, 1967



APPENDIX 1. Continued.

Species	Response Type	Conditions giving rise to reversion	Characteristics of reverted plants	Reference
<u>Cosmos bipinnatus</u> L.	SDP	9 short days followed by long days.	Foliaceous bracts, and then internode below the inflorescence. Inflorescence more or less vegetative; some plants reverted to vegetative growth after initiating 1 cycle of bracts.	Greulach, 1942
<u>Gylcine max</u> (L.) <u>Merrill.</u> cv. <u>Biloxi.</u>	SDP	17 short days followed by 22 long days.	Axillary primordia initiated in inductive period do not develop, plants gradually acquire vegetative characteristics. Cambial activity resumes on reversion.	Struckmeyer, 1941.
<u>Hyoscyamus niger</u>	LDP	Plants grown in short days. Given 3 long days, returned to short days.	Rosette of leaves formed on reversion, intermediate apex type.	Seidlová & Juráková-Štichová, 1965.



APPENDIX 1. Continued.

Species	Response Type	Conditions giving rise to reversion	Characteristics of reverted plants	Reference
<u>Kalanchoe blossfeldiana</u> <u>Poellniz</u>	SDP	Low inductive stimulus.	Vegetative inflorescence formation from below the single flower. Leafy bracts.	Harder, 1948
<u>Melilotus albus</u>	LDP	Near critical photoperiod.	Large foliaceous bracts in the inflorescence.	Kasperbauer Gardner and Loomis, 1962
<u>Nicotiana glutinosa</u> L.	DNP	Induction in light of normal intensity (12000 lx), transfer to low intensity (1500 lx) at young inflorescence stage.	Return to leaves with vegetative branches, zone of leafy bracts and aborted flowers on transition.	Diomaiuto-Bonnand, 1969
<u>Perilla crisp</u> (Thumb) <u>Tanaka</u> . (Green-leaved and red-leaved types)	SDP	20 short days (inductive), return to long days for 2 months.	Terminal meristem (T.M.) returns to production of leaves and vegetative branches rather than bracts and axillary flowers <u>via</u> a series of intermediate stages.	Jacobs and Raghavan, 1962 Zeevaart, 1969

Continued.



APPENDIX 1. Continued.

Species	Response Type	Conditions giving rise to reversion	Characteristics of reverted plants	Reference
<u>Perilla crispa</u> (Green-leaved and red-leaved types) Continued		<p>Occurs because T.M. never forms a terminal flower itself.</p> <p>Explanation: Leaves induced by short days retain induced state in long days; new leaves/bracts produced in long days non-induced and take over control of growing point. Evidence, however, that induced leaves do NOT retain this state in long days.</p>		<p>Nougarède et al., 1964</p> <p>Zeevaart, 1958</p> <p>Lam and Leopold, 1961.</p>
<u>Petasites hybridus</u>	SDP	Bisection of apex, removal of the bracts, or puncturing the apical dome, just after initiation of bract primordia.	Reversion to vegetative growth - primordia that would have been bracts developed as leaves. After late transition stage no reversion.	Wardlaw, 1963
<u>Salvia splendens</u> var. <u>Feu de la Saint Jean</u>	LDP	<15 long days before return to short days of 7 hrs. 23 long days followed by 8 hrs short days.	Leaves become bract-like, axils give rise to vegetative branches /aborted flowers. Return to normal leaf production.	Crawford, 1961



APPENDIX 1. Continued

Species	Response Type	Conditions giving rise to reversion	Characteristics of reverted plants	Reference
<u>Scabiosa</u> <u>ukranica</u>	LDP	Transfer from long days to short days.	Flowers already present on transfer back to short days develop normally. Those axillary buds not flowering on transfer initiate leaves instead of flower parts.  Axillary buds of first order form a whorl of bracts and then abort. Axillary buds of second order form a whorl of bracts and then return to vegetative growth.	Chouard, 1950a         Chouard, 1950b
<u>Sinapis alba</u> L.	LDP	13-20 long days (inductive); 40-60 short days; return to long days to reveal reversion.	Elongated inflorescence (I) axis, lower flowers of I aborted, leaves with vegetative branches above I. Transition zone between I and vegetative parts characterised by the presence of leafy bracts and some proliferous/virescent flowers.	Bernier & Dath, 1962       Bagnard, 1980a and b 1983

Continued



APPENDIX 1. Continued

Species	Response Type	Conditions giving rise to reversion	Characteristics of reverted plants	Reference
<u>Sinapis alba</u> L. Continued		Long days (inductive) followed by short days with 1 h night breaks. Low light intensity during inductive phase increases % plants subsequently reverting.	I elongation, rosette of leaves above I.  Terminal meristem of plants having undergone reversion has appearance of an inflorescence meristem.	Arnal, 1966  Bagnard, Bernier & Arnal, 1972
<u>Trifolium repens</u>	SLDP	6 days continuous light, return to short days.	Return to production of vegetative axillary branches.	Thomas, 1961c, 1962
<u>Xanthium strumarium</u> L.	SDP	Decapitation at 3rd node following 3 short day treatment. More inductive cycles - must decapitate more times to bring about reversion.	Axillary branches grow out in vegetative state, rather than floral.	Lam and Leopold, 1960



APPENDIX 2



APPENDIX 2

A) Composition of Edinburgh formulation of G.C.R.I.

Potting compost number 1. (3:1 Peat:Sand mix)

Parts by volume:     3             Sphagnum Peat  
                         1             Lime-free Sand,

To which was added, per cubic metre:

0.40 kg   Ammonium nitrate  
1.50 kg   Superphosphate  
0.75 kg   Potassium nitrate  
2.25 kg   Ground limestone  
2.25 kg   Dolomitic limestone  
0.38 kg   Fritted trace elements

FTE 253A  
(SAI Horticultural Ltd),  
i.e. not WM 255 as per  
Machin and Scopes).

From: Machin and Scopes, 1978

B) Composition of Hoagland's solution as used for liquid  
feed. (Hoagland and Arnon Number 1)

In 1 litre of solution:

5 ml   1M  $\text{KNO}_3$   
5 ml   1M  $\text{Ca}(\text{NO}_3)_2$   
5 ml   0.025% EDTA  
10 ml   1/10 strength Micronutrients\*  
5 ml   0.4M  $\text{MgSO}_4$   
5 ml   0.2M  $\text{KH}_2\text{PO}_4$

\*Full Strength Micronutrients:

2.86 g  $\text{dm}^{-3}$   $\text{H}_3\text{BO}_4$   
1.81 g  $\text{dm}^{-3}$   $\text{MnCl}_2 \cdot \text{H}_2\text{O}$   
0.22 g  $\text{dm}^{-3}$   $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$   
0.08 g  $\text{dm}^{-3}$   $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$   
0.09 g  $\text{dm}^{-3}$   $\text{H}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$

From: Hoagland and Arnon, 1950



APPENDIX 3



## Changes in Apical Growth and Phyllotaxis on Flowering and Reversion in *Impatiens balsamina* L.

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### ABSTRACT

When plants of *Impatiens balsamina* L. were subjected to 5 short days and then re-placed in long days, they began to form a terminal flower and then reverted to vegetative growth at this terminal shoot apex. The onset of flowering was accompanied by an increase in the rate of initiation of primordia, an increase in the growth rate of the apex, a change in primordium arrangement from spiral to whorled or pseudo-whorled, a lack of internodes, and a reduction in the size at initiation of the primordia and also of the stem frusta which give rise to nodal and internodal tissues. On reversion, parts intermediate between petals and leaves were formed, followed by leaves, although in reverted apices the size at initiation and the arrangement of primordia remained the same as in the flowering apex. The apical growth rate and the rate of primordium initiation were less in the reverted apices than in floral apices but remained higher than in the original vegetative apex. Since the changes in apical growth which occur on the transition to flowering are not reversed on reversion, the development of organs as leaves or petals is not directly related to the growth rate of the apex, or the arrangement, rate of initiation or size at initiation of primordia.

Key words: *Impatiens balsamina* L., flower reversion, evocation, phyllotaxis, shoot meristem.

### INTRODUCTION

Evocation consists of the events in the shoot apex which lead to flowering (Evans, 1969). To understand how flowering is controlled, it is necessary to know how these events are linked to the subsequent initiation of flower parts. However, the mechanism by which an evocational event has its effect is unknown, because it is thought of as leading to commitment to flowering, and the nature of this commitment step is unknown. The time of commitment to flower has been established in plants such as *Sinapis alba*, in which a 'point of no return' can be demonstrated about 44 h after the start of the inductive long day (Kinet *et al.*, 1971). In plants in which reversion of the flower meristem to vegetative growth can occur after flower formation has started, it is obvious that there is no 'point of no return' before flower development begins. This suggests that in such plants there is no commitment, and we can then ask whether there is a direct relationship between the events of evocation and the events of flower formation in these cases. Furthermore, it should be possible to see whether reversion to vegetative growth involves reversal of all preceding evocational changes, or whether the apex that results is partially evoked. The response of a partially evoked apex to further induction should allow us to pinpoint which subsequent evocational changes correspond to particular events of flower formation.

Plants showing reversion have not been used to study evocation because the sporadic nature of reversion in the species in which it has been observed has, until now, not allowed an experimental approach. For this to be possible reversion must be sufficiently consistent and predictable in all or most of the plants. In order to produce an experimental system a preliminary study was made of some species in which reversion of flowers occurs –



*Pharbitis nil* (King and Evans, 1969), *Anagallis arvensis* (Brulfert and Chouard, 1961) and *Impatiens balsamina* (Simon, 1973). Of these, only *I. balsamina* could be made to revert from flowering in a sufficiently predictable manner to allow experimentation.

*I. balsamina* is a short-day plant which reverts to leaf production if transferred back to long days (LD) during flower development (Krishnamoorthy and Nanda, 1968; Debraux and Simon, 1969). It forms zygomorphic lateral flowers and also a large actinomorphic terminal flower with many floral parts. It is easiest to study flowering and reversion at this terminal meristem. In this paper we first describe the development of experimental regimes for the induction of flowering and for reversion in this terminal flower of *I. balsamina*. Secondly, we compare, in flowering and reverting plants, the changes in apical growth rates and in the arrangement, size and rate of initiation of primordia, in order to establish whether such changes, which are usually associated with flowering (Bernier, Kinet and Sachs, 1981) are necessarily linked to the production of flower parts in *I. balsamina*. If they are, they would presumably be reversed on reversion, and would therefore warrant particular attention as possible essential, causal, events for the initiation of flower parts.

The results show that the reverted apex is not the same as either the original vegetative apex or the flowering apex; nor are the morphological changes characteristic of evocation in *I. balsamina* reversed on reversion.

## MATERIALS AND METHODS

### *Growth of the Plants*

Mixed seed of *Impatiens balsamina* L. (cv. Dwarf Bush Flowered) was obtained from Wm K. McNair, Portobello, Edinburgh, U.K. Batches of seeds were imbibed on moist filter paper in LD for 65 h. Then the seeds with emerged radicles 3–4 mm long were sown at a depth of 1 cm in moist 3:1 peat:sand mix (G.C.R.I. No. 1 Formulation). Twenty-four seeds were sown per 37 × 23 × 6 cm holed tray, and watered until the soil was thoroughly moist. The trays were placed in LD of 24 h, consisting of 8 h light, provided by Philips 65–80 W white fluorescent tubes supplemented by tungsten bulbs (total photosynthetic photon flux density 275–285  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at the top of the plants on day 0; see below), followed by 16 h light of low photosynthetic photon flux density (6  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at the top of the plants on day 0) provided by tungsten bulbs. Temperature was  $22 \pm 1$  °C, and relative humidity 55–65 per cent. The soil was kept moist by application of 200 ml distilled water per tray every day, except every third day when 200 ml full-strength Hoagland's solution (Hoagland and Arnon, 1938) was supplied.

Seven days after sowing the young seedlings were selected for developmental uniformity; all those in which the first leaf was not 4–8 mm long were discarded. The day on which this selection was carried out marked the beginning of treatments in inductive short days (SD), and was designated day 0. A sample of five or six plants was taken after selection to establish the total number of leaves plus primordia present. Plants to be induced to flower were transferred to SD in which the 8 h light period was followed by 16 h dark, at  $21 \pm 1$  °C. Normal flowering controls remained in SD until dissection 6–7 weeks later. About 90 per cent of the plants formed a terminal flower; the remaining 10 per cent formed flowers only at the axillary meristems and could be distinguished by their modified phyllotaxis and/or the presence of axillary flowers. Only the plants forming terminal flowers were used in the analyses described here.

Reversion was brought about by transfer back to LD after varying numbers of SD; vegetative controls were plants that remained in LD from day 0. About a quarter of the plants which remained in LD showed either an anomalous form of terminal flower formation, or flowering at the axillary meristems only. Since these plants could be



distinguished from vegetative plants at an early stage, they were excluded from the analyses described here.

### *Numbers of Leaves plus Primordia*

Groups of not less than five plants were sampled at intervals during the experimental period, dissected out under a dissecting microscope, and the total number of leaves plus primordia (excluding the cotyledons) counted on each plant. Values of total number of leaves plus primordia obtained during sampling of plants for the analyses described below were also included. Rates of primordium initiation per day were calculated as described in the results section.

### *Developmental Stages*

Plants were grouped into developmental stages according to the total number of leaves plus primordia present at the time of sampling, in all the growth analyses to be described. These stages (see Tables 4–6) were chosen to correspond to distinct phases of development in all treatments, and allowed comparison between the different treatments, and different phases of development during the same treatment.

### *Distances of Primordia below the Apical Surface*

The shoot apex of *I. balsamina* has the shape of a flat-topped cone; the flat top bears the young primordia and will be referred to as the apical surface. The distances of primordia below the apical surface were measured to provide an estimate of the vertical relative growth rate per plastochron of the apex, and of the size of the stem frustum on initiation. This frustum includes the stem tissue adjacent to the primordium, and the stem tissue between the abaxial insertion point of the primordium and the adaxial insertion point of the next oldest primordium. These tissues give rise to the node and internode, respectively, in the mature plant. To provide these measurements three to six plants were taken on days 8, 10 and 12 following selection, the nine oldest leaves and the cotyledons removed, and the remaining shoot plus primordia fixed in formalin–acetic acid–50 per cent ethanol (1:1:18 by volume) for at least 24 h. The fixed tissues were dehydrated through an ethanol series and embedded in wax. Serial transverse sections, 5  $\mu\text{m}$  thick, were stained with 0.5 per cent Toluidine Blue O (O'Brien and McCully, 1981) or by the method of Sharman (1943). From these sections the developmental stage of each plant was first determined by counting the number of primordia in the first transverse section to graze the surface of the shoot apex, and adding to this the nine leaves removed before embedding, to give the total number of leaves plus primordia.

The distances of the successive leaves or primordia below the apical surface were then measured by recording, for each, the section in which connection between its base and the stem was half complete. Where the succession was in doubt (e.g. in pseudo-whorls), it was assumed that the primordia were initiated with successive angular separations of approximately  $137^\circ$ . In some cases (particularly in flowering or reverting plants) shoot growth was not straight, resulting inevitably in some skewed sections. In these cases the distances of the primordia below the apical surface were measured in relation to the youngest primordium on the same side of the apex.

The number of primordia on the apical surface was also estimated from these measurements. Primordia with a mean transformed distance,  $\log_e(x \mu\text{m} + 1)$ , of less than  $\log_e(5 \mu\text{m} + 1)$  below the apical surface were defined as being on the apical surface (see also Fig. 3).



### *The Plastochron Ratio*

The plastochron ratio – the ratio between the transverse distance of a primordium from the centre relative to that of the next primordium to be produced (Richards, 1951) – was measured on photographs of the shoot apex, taken with a Cambridge Stereoscan 250 scanning electron microscope (SEM). Six plants were taken on days 8, 10 and 12 following selection, the cotyledons and the nine oldest leaves were removed, and the remaining shoot plus young parts was frozen in liquid nitrogen. These stem tips were each placed in a screw-topped tube containing a small amount of drying agent (molecular sieve type 4A; BDH) and a large excess of absolute methanol, at  $-80^{\circ}\text{C}$ . They were then freeze-substituted in absolute methanol for 2 weeks at  $-80^{\circ}\text{C}$ , 1 week at  $-40^{\circ}\text{C}$ , 1 d at  $-20^{\circ}\text{C}$ , 15 h at  $4^{\circ}\text{C}$  and 2 h at room temperature (method modified from Robards, 1978). After transfer to fresh absolute methanol the tissues were passed through a methanol-acetone series to absolute acetone. Following critical-point drying with carbon dioxide, the apices were mounted on stubs, and the older primordia were carefully removed to reveal the apical surface, whilst leaving clear outlines of the positions of the primordial bases around the apex. The dissected apices were gold-coated and viewed from directly above in the SEM. Photographs were taken at successive magnifications, so as to include all the young primordia and the bases of those removed during dissection. The outlines of the primordia were traced from the photographs, and the centre of each was estimated using circles of appropriate size and equalizing parts of the primordium bases outside the circles.

The plastochron ratio ( $r$ ) can be measured most easily by the method of Maksymowych and Erickson (1977), in which only the order of initiation of primordia need be known. In vegetative plants, and for those primordia on flowering and reverting meristems initiated before and during the transition to flowering, in which the order of initiation could be determined from the genetic spiral, this method was therefore used. The order of initiation of primordia becomes difficult to establish objectively after the transition to flowering, so the plastochron ratio was estimated from these primordia in the following way. Firstly, the position of the apical centre was fixed from the intersection point of the radii from the older primordia whose order of initiation was known, and whose distances from the apical centre could be calculated by the method of Maksymowych and Erickson (1977). The distances from this centre of those primordia initiated after the transition to flowering were then measured. The order of their initiation was taken to be a function of their radial distances, and the plastochron ratios calculated by the method of Richards (1951). The areas of primordia on initiation, relative to the area of the free apical surface, and area relative growth rates per plastochron ( $2 \log_e r$ ) of the apex, were calculated from the mean plastochron ratios derived from primordia nearer the centre than, and including, the first primordium below the apical surface.

### *Area and Vertical Relative Growth Rates of the Apex*

These were obtained as area and vertical relative growth rates per plastochron respectively, multiplied by the rate of initiation of primordia per day.

## RESULTS

### *Morphological Characteristics of Flowering and Reversion*

Examination of over 200 plants has shown that in continuous SD the terminal flower is formed as follows. At the time of transfer to SD about eight or nine leaves plus primordia are present. Part 9 or 10 (numbering from the first leaf upwards) is usually



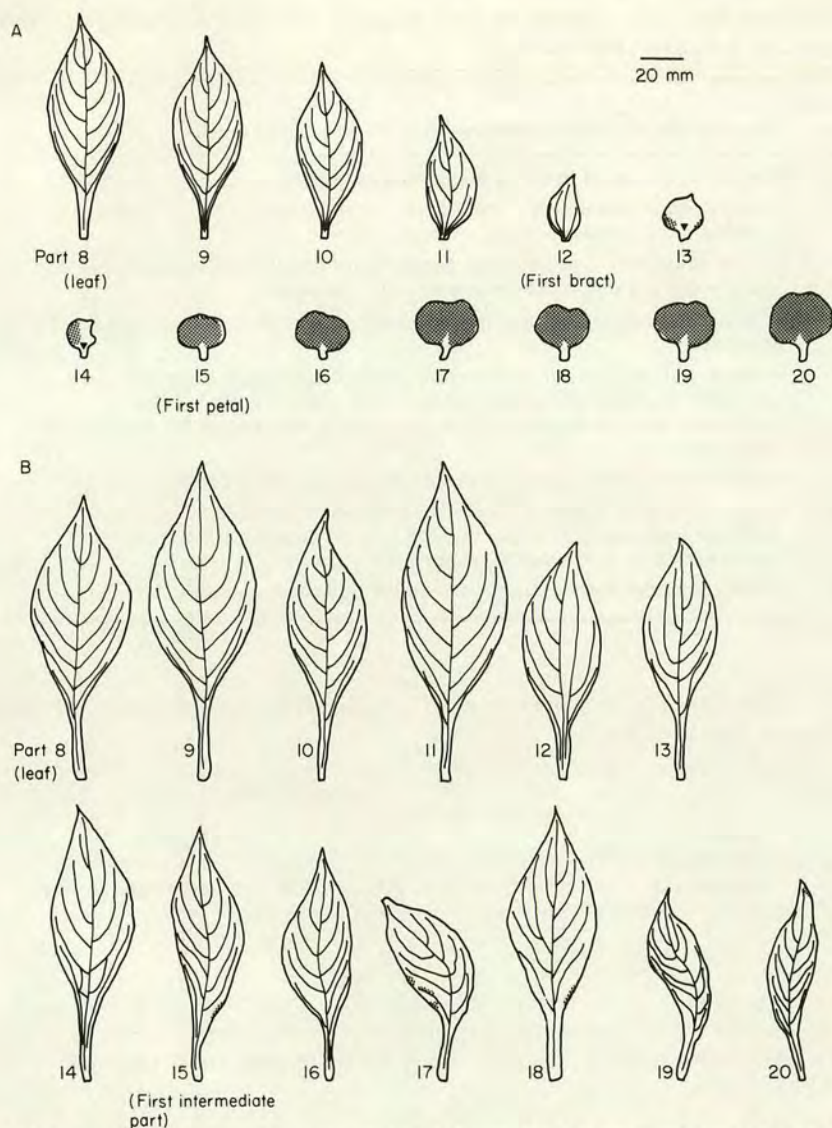


FIG. 1. Changes in morphology of successively initiated parts (A) of the normal flower (SD) and (B) of the reverted flower (5 SD + LD), R4 reversion type, of *I. balsamina*. Successive parts were removed from these mature structures and the outlines and clearly visible venation of each traced. Areas with pigment typical of petals are shaded. ▼ indicates the presence of a spur. Part 1 = the lowest leaf on the plants.

the first to show differences from normal leaves, since this and all subsequent parts lack axillary structures, and one or both of the basal pair of second-order veins are not fused to the main vein in the lamina, but retain an independent identity into the petiole (Fig. 1A). Parts 10–14 usually show increasing degrees of such modified venation, the second pair of second-order veins being affected in a similar way to the basal pair. These parts also show progressive reduction in size compared with normal leaves, may have small



TABLE 1. *Characteristics of reversion types obtained as a result of transfer of I. balsamina plants back to LD after three to 10 SD*

Reversion type	Characteristics of plants on dissection 6 weeks after return to LD
R0	Plants show no sign of flowering and subsequent reversion
R1	The only sign of flowering is a zone of leaves with no, or modified, axillary structures (the reversion zone)
R2	Zone of leaves with virescent lateral flowers of the terminal inflorescence, above which is a zone of leaves with no axillary structures
R3	As above, but with reduction of internodes between the leaves with no axillary structures
R4	As above, but the leaves of the reversion zone not separated by internodes, and some have modified venation and/or areas with pigment typical of petals; subsequent leaves in a whorled or pseudo-whorled arrangement are separated by internodes
R5	As above, but a zone of petals occurs before return to leaf production
R6	As above, but with a zone of stamens after the petals, followed by a return to leaves, or occasionally by primordia, either leaves or petals, whose exact nature cannot be determined by inspection
R7	As above, but the zone of stamens is followed by a return to petals

TABLE 2. *Effect of different numbers of SD on the proportion of plants of I. balsamina showing each reversion type*

Number of SD before return to LD	Number of plants					Flowering and not reverting	Total
	R4	R5	R6	R7			
5	13	2	1	1		6	23
7	4	7	3	2		11	27
8	2	11	1	1		11	26
10	2	3	1	3		9	18

R4, R5, ... = reversion type 4, 5... (no plant showed R0–R3 reversion types). Combined data from two experiments.

areas of coloured pigment characteristic of petals, generally lack a petiole, and may also have a spur. Those parts which are less than 50 mm long when mature, and which lack a petiole and/or have a spur, are referred to as bracts. The first petal is usually part 15, and is defined as the first part lacking a spur which has more than 50 per cent of its area pigmented. Successive petals become more pigmented until the maximum amount of pigmentation (about 85 per cent of the petal) is attained by about part 17. Petals are usually followed by stamens and carpels (Debraux and Simon, 1969), although in some plants carpels, or stamens and carpels, may not be formed. In these cases petals continue to be initiated instead.

The modified leaves and bracts are initiated during the first five SD. Petals are initiated from the fifth SD onwards, until the formation of the first stamen (about part 34–35) on about the 12th SD. Carpel formation begins on about the 19th SD (about part 56–57).



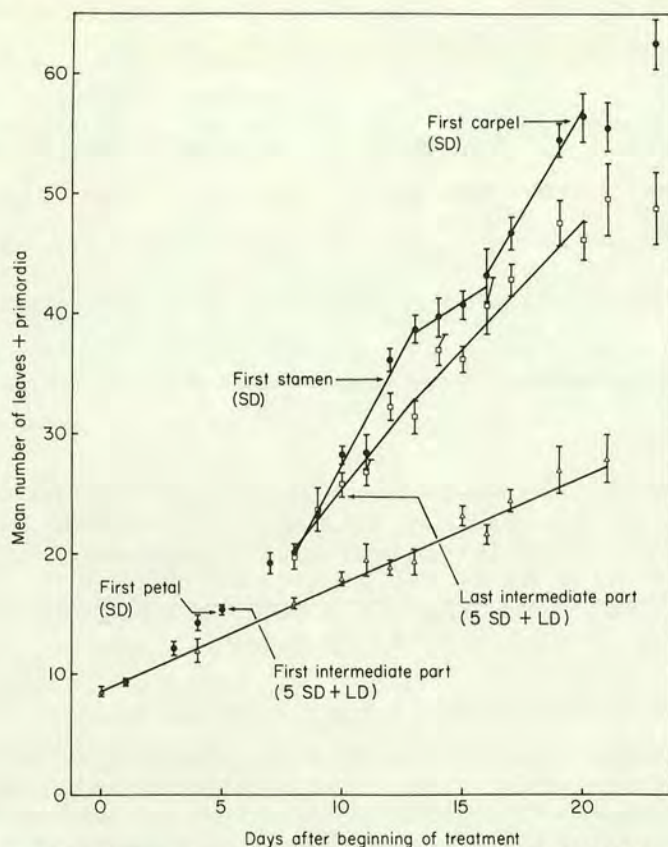


FIG 2. Change of primordium number in vegetative (LD) ( $\Delta$ ), flowering (SD) ( $\bullet$ ), and reverting (5 SD + LD) ( $\square$ ), plants of *I. balsamina*. Combined data from 10 experiments. Mean values  $\pm$  1 s.e. are presented. Full lines are those given by regression equations. Times at which changes in initiation rate occur were determined by inspection for the SD treatment. To allow comparison of the rates of initiation from day 8 to day 13 in SD and 5 SD + LD, regression lines were fitted from day 8 to day 13, and from day 13 to day 20, in the 5 SD + LD treatment. Values of the regression coefficients are given in Table 3.

Transfer of plants from SD back to LD, before initiation of the terminal flower is complete, causes this flowering terminal meristem to revert to leaf production in most cases. The types of reversion obtained (Table 1) range from R 1, in which the only sign of an effect of SD is a zone of leaves with no axillary structures, through R 4, in which a zone of intermediate parts (leaves with some pigmented areas – see Fig. 1 B) is followed by a return to leaf production in modified arrangement, to the R 6–R 7 types. In both R 6 and R 7 petals and stamens are formed and are followed by leaves in R 6 or petals in R 7.

How far flower formation progresses before reversion depends on the number of SD the plant has received (Table 2). With an increased number of SD there is a decreased proportion of reverting plants of the R 4 type, and an increased proportion of R 5 to R 7 types. The 5 SD treatment causes the most uniform reversion response (Table 2). In the R 4 type that results from this treatment in most cases, the lack of internodes in the reversion zone and the presence of intermediate parts is evidence that flowering has begun,



TABLE 3. *Rates of primordium initiation during different phases of development of I. balsamina plants growing in LD (vegetative), SD (flowering), and 5 SD+LD (reverting)*

Treatment	Type of apex	Days after beginning of treatment	Rate of initiation of primordia $\pm$ 1 s.e. (primordia per day)
LD	Vegetative	0-21	$0.9 \pm 0.03$
SD	Flowering	8-13	$3.8 \pm 0.3$
		13-16	$1.3 \pm 0.2$
		16-20	$3.4 \pm 0.3$
5 SD+LD	Reverting	8-13	$2.5 \pm 0.3$
		13-20	$2.2 \pm 0.3$

Rates of initiation were estimated as the regression coefficients from regression lines fitted to the data of Fig. 2.

but leaf initiation and internodal development resume more quickly than in the R5-R7 reversion types. For these reasons five SD were used to bring about reversion in the experiments to be described. In these experiments, although most of the plants in the 5 SD treatment will be R4 reversion types, the data will inevitably include other reversion types and non-reverting plants. However, those statements concerning the morphology of reversion refer to the R4 type exclusively.

#### *Changes in Apical Growth and Phyllotaxis on Flowering and Reversion*

**Rates of Primordium Initiation** The rate of primordium initiation during vegetative growth in LD is constant (Fig. 2) and is  $0.9$  primordia  $d^{-1}$  (Table 3). In continuous SD the rate of primordium initiation increases from the vegetative rate during the first eight SD, and between days 8 and 13, when petals and the first stamens are being initiated, increases further to  $3.8$  primordia  $d^{-1}$ . During the initiation of stamens (days 13-19) the rate declines to  $1.3$  primordia  $d^{-1}$  and then increases to about  $3.4$  primordia  $d^{-1}$  before carpel initiation begins on about day 19 (Fig. 2; Table 3). The most rapid rate of primordium initiation during flowering therefore accompanies the initiation of petals and the earliest and latest stamens.

In plants given 5 SD+LD the rate of primordium initiation has increased from the vegetative value by day 8, as in flowering plants. After day 8 it does not increase to the same degree as in SD (Table 3), since from day 10 onwards the mean number of leaves plus primordia from plants in 5 SD+LD is consistently lower than in SD plants (Fig. 2). The rate of primordium initiation in 5 SD+LD is  $2.5$  primordia  $d^{-1}$  between days 8 and 13, and  $2.2$  primordia  $d^{-1}$  between days 13 and 20 (Table 3). Therefore, when the reverting plants begin to make leaves again (about day 10) the rate of primordium initiation is not quite as rapid as during petal initiation in SD, but is more than twice as rapid as during leaf initiation in vegetative plants in LD. Clearly, there is not a particular rate of initiation associated with leaf production, and reversion does not result in a return to the original vegetative (LD) rate.

**Divergence Angles** Vegetative plants always have spiral phyllotaxis with a mean divergence angle of  $137.9^\circ$  (1 s.e. =  $0.6$ ,  $n = 76$ ). At about part 21, whorls or pseudo-whorls, each of about six parts, are formed in both the SD and the 5 SD+LD treatments, even though petals are formed in SD, but intermediate parts in 5 SD+LD. A pseudo-whorled or whorled arrangement persists during subsequent flowering in SD and



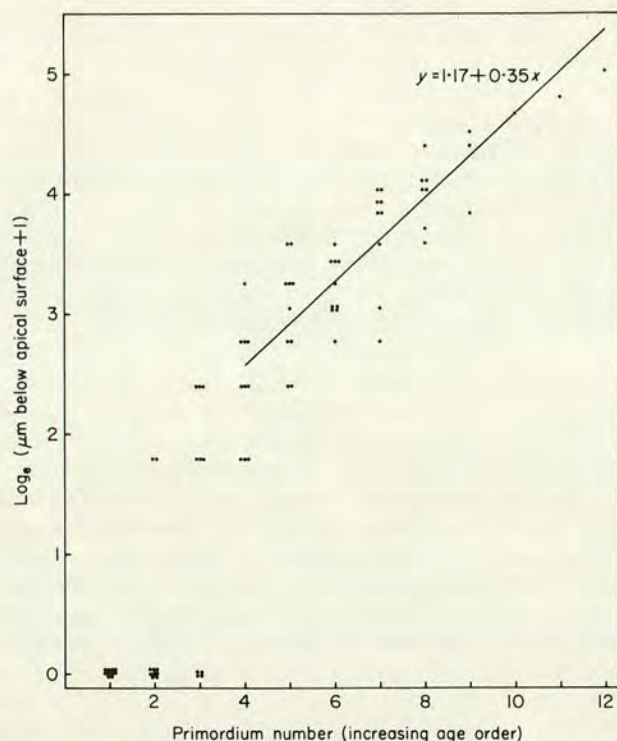


FIG. 3. Estimation of the length of a frustum on initiation: illustration of the method used for vegetative (LD), flowering (SD), and reverting (5 SD + LD) plants of *I. balsamina* at different developmental stages.  $\text{Log}_e(x \mu\text{m below the apical surface} + 1)$  of the insertion points of primordia from 10 plants growing vegetatively in LD is plotted as a function of primordium number (the youngest primordium visible in the transverse section grazing the apical surface = P1). The mean transformed distances of P1–P3 below the apical surface are less than  $\text{log}_e(5 \mu\text{m} + 1)$ ; to allow comparison with flowering and reverting apices these primordia are defined as being on the apical surface. The regression line was fitted for primordia P4–P12, and the regression coefficient estimates  $\text{log}_e v$ , where  $v$  is the relative distance of any two successive primordia from the apical surface.  $\text{Log}_e v$  is an estimate of the length of the frustum on initiation, relative to the tissue distal to the first primordium below the apical surface (P4 in this case).

also on reversion to leaf initiation in 5 SD + LD – there is no immediate return to the spiral arrangement found in LD (vegetative) plants.

**Area of the Apical Surface and Sizes of Primordia at Initiation (Table 4)** In vegetative, flowering and reverting plants the youngest primordium is initiated at about the same distance from the apical centre; hence the bare area of the apical surface,  $\pi \times (\text{mean radial distance of the youngest primordium from the apical centre})^2$ , remains constant at about  $9 \times 10^3 \mu\text{m}^2$  at all the stages measured. The total area of the apical surface,  $\pi \times (\text{mean radial distance of the first primordium below the apical surface})^2$ , also remains constant at about  $41 \times 10^3 \mu\text{m}^2$  in the three treatments. This implies that the area of the annulus bearing the primordia on the apical surface is also constant, and this is what is found. However, the number of primordia occupying this area increases to about the same extent in both the flowering and the reverting apices, so that there are two to three times as many primordia on the apical surface as on that of a vegetative plant. The implication is that the primordia must be smaller in the flowering and in the reverting apices. Using



the plastochron ratio ( $r$ ), the area of a primordium at initiation, relative to the area of the apex, can be calculated as  $2 \log_e r$  (Richards, 1951). In both the flowering and the reverting plants the area of a primordium at initiation relative to the area of the apical surface, calculated in this way, decreases significantly from that in the vegetative apex. The absolute area of a primordium on initiation also decreases on flowering and remains lower on reversion. Confirmation of this trend was obtained when the area of primordia on initiation was estimated from regression lines fitted to graphs of  $\log$  (basal area of the primordium) against primordium age in plastochrons.

On flowering there is therefore no change in the area of the apical surface, but there is a reduction in the absolute area of the primordia initiated. This reduction in size is maintained when the plant reverts to leaf initiation, indicating that the size of the primordium at initiation does not determine its developmental fate.

*Lengths of Stem Frusta on Initiation (Table 5)* Just as the area of a primordium at initiation (relative to the area of the apex) is  $2 \log_e r$ , where  $r$  is the plastochron ratio, by analogy the length of the frustum on initiation is  $\log_e v$ , where  $v$  is the relative vertical distance of successive primordia from the apical surface. As Richards (1951) says of the plastochron ratio, 'one may define how one pleases the size of the circle to represent the mean area of the apex, but once this is defined the "primordial area" is automatically defined also... In the present case the area taken to represent the "bare" apex at its mean size is that circle on whose circumference the geometrical centres of the primordia *make their first appearance*' (our italics). Similarly, we define the frustum as being initiated when it can first be measured (i.e. between the first and second primordia below the apical surface), equivalent to defining the primordium as being initiated at the time it can first be seen. Just as the tissue generating the primordium is the tissue nearer the centre of the apex than the first primordium, the tissue generating the frustum is the tissue distal to the first frustum. The length of the tissue distal to the first frustum has already been defined as  $\log_e(5 \mu\text{m} + 1)$ , because this is the level at which a primordium is defined as first being below the apical surface. The procedure used to estimate the length of the frustum on initiation (relative to the tissue generating it) is illustrated in Fig. 3.

The length of the frustum on initiation relative to the length of the tissue distal to it, measured in this way, decreases in the flower compared to the vegetative apex, and is also smaller in the reverted apex. The tissue distal to the first frustum is  $6\text{--}11 \mu\text{m}$  long; the absolute length of the frustum on initiation, calculated as the product of this and the relative length, is also lower in the flowering and reverted plants than in the vegetative plants. The larger frustum on initiation in the vegetative plants, compared with the flowering plants, is correlated with the length of internodes at maturity: vegetative plants have long internodes separating their leaves, whilst flowering plants have no visible internodes separating the parts of the mature flower. Reverted plants at the 'leaf' stage in the 5 SD+LD treatment have initiated 30–34 parts, 10 of which are on the apical surface (Table 4). Therefore, estimates of frustum length at initiation, in plants at this stage, are made from parts 10–24. All these parts lie within the reversion zone of mature R4 plants, since the first internode above this zone occurs above part number 24.4 (1 s.e. = 0.7,  $n = 39$ ). Consequently, the similar values for frustum length at initiation (relative or absolute) in SD and 5 SD+LD are consistent with the hypothesis that a smaller frustum length at initiation may result in a lack of internode development at maturity.

On reversion, then, at the time that intermediate parts and the first leaves are initiated, the apex initiates frusta of the same size as those found in plants flowering normally in SD. The formation of intermediate parts and leaves is therefore not accompanied by reversion of the apex to the vertical growth pattern found in vegetative (LD) plants. Further measurements would be needed to determine whether such a change eventually occurs to give rise to the internodes seen after the reversion zone.



TABLE 4. Area of the apical surface and areas of primordia on initiation during different phases of development of vegetative (LD), flowering (SD), and reverting (5 SD + LD) plants of *I. balsamina*

Treatment	Type of apex	Stage of development (total no. of leaves + primordia)	Number of plants per sample	Mean distance from the apical centre of		Area of annulus bearing primordia on apical surface ( $10^3 \times \mu\text{m}^2$ ) ( $\pm 1 \text{ s.e.}$ )†	Number of primordia on the apical surface‡	Area of primordium at initiation relative to area of apical surface ( $2 \log_e r$ )§	Absolute area of primordium at initiation ( $10^3 \times \mu\text{m}^2$ )	Absolute area of primordium at initiation (intercept at age 0 of regression of log (basal area of primordia) against primordium age in plastochrons) ( $10^3 \times \mu\text{m}^2$ )
				The youngest primordium ( $\mu\text{m}$ )*	The first primordium below the apical surface ( $\mu\text{m}$ )*					
LD	Vegetative	Leaf	10	59.5	112.1	$28.3 \pm 2.7$	3	0.41	4.7	3.6
SD	Flowering	Mid-petal (21–29)	3	48.9	103.2	$29.1 \pm 9.9$	7	0.23	1.8	1.3
		Late petal (30–38)	7	44.6	116.8	$37.8 \pm 5.4$	8	0.26	1.7	1.6
5 SD + LD	Reverting	Intermediate part/leaf (21–29)	7	60.5	112.4	$27.5 \pm 3.9$	7	0.20	2.4	1.4
		Leaf (30–34)	5	49.1	125.1	$43.3 \pm 6.9$	10	0.20	1.6	1.6

\* Geometric means presented; analysis of variance on log-transformed data n.s.

† Analysis of variance on all five treatment stages: n.s.

‡ Data obtained from serial transverse sections; see Table 5 for number of plants per sample.

§ Analysis of variance on all five treatment stages: LD plants significantly different from remaining plants at  $P = 0.005$ . All other data in this table were obtained from SEM photographs.



TABLE 5. *Lengths of stem frusta on initiation during different phases of development of vegetative (LD), flowering (SD), and reverting (5 SD + LD) plants of I. balsamina*

Treatment	Type of apex	Stage of development (total no. of leaves + primordia)	Number of plants per sample	Mean distance of the first primordium below apical surface from apical surface (length of the tissue generating the frustum) ( $\mu\text{m}$ ) (1)*	Length of frustum at initiation relative to the length of tissue generating the frusta ( $\log_e v$ ) ( $\pm 1$ s.e.) (2)	Absolute length of the frustum at initiation [(1) $\times$ (2)] ( $\mu\text{m}$ )
LD	Vegetative	Leaf	10	10.2	$0.35 \pm 0.02^\dagger$	3.6
SD	Flowering	Mid-petal (21–29)	2	8.8	$0.31 \pm 0.06$	2.7
		Late petal (30–38)	4	6.7	$0.17 \pm 0.01^\ddagger$	1.1
5 SD + LD	Reverting	Intermediate part/leaf (21–29)	3	6.3	$0.27 \pm 0.02$	1.7
		Leaf (30–34)	4	7.2	$0.22 \pm 0.02^\ddagger$	1.6

\* Geometric means presented; analysis of variance on log-transformed data n.s.

† Significantly different from ‡ at  $P = 0.001$ .TABLE 6. *Area and vertical relative growth rates ( $d^{-1}$ ) of the apical surface and frusta during different phases of development of vegetative (LD), flowering (SD), and reverting (5 SD + LD) plants of I. balsamina*

Treatment	Type of apex	Stage of development (total no. of leaves + primordia)	Area relative growth rate ( $2 \log_e r \times \text{rate of primordium initiation}$ )*	Vertical relative growth rate ( $\log_e v \times \text{rate of primordium initiation}$ )*
LD	Vegetative	Leaf	0.37	0.32
SD	Flowering	Mid-petal (21–29)	0.87	1.18
		Late petal (30–38)	0.99	0.65
5 SD + LD	Reverting	Intermediate part/leaf (21–29)	0.50	0.68
		Leaf (30–34)	0.50	0.55

\* Since both area and vertical relative growth rates are derived from values (see Tables 3, 4 and 5) which have error terms, standard errors cannot be calculated for the values presented.



*Area and Vertical Relative Growth Rates of the Apex (Table 6)* At the time of petal initiation both the area and the vertical relative growth rates of the apex have increased about two- to threefold. In the reverting apex the area and vertical relative growth rates are intermediate between the rates in vegetative and flowering apices. The higher growth rate is therefore specific to petal initiation, since it does not occur during initiation of parts of the same plastochron age in the reverting apex. Even so, the reverted apex grows at a higher rate than the vegetative (LD) apex, indicating that reversion to leaf initiation does not involve complete reversal of the increase in growth rate accompanying flowering.

## DISCUSSION

Although on reversion leaves are initiated instead of petals, the arrangement and size of the primordia at initiation are the same as in the flowering plant, and are therefore not necessarily linked to the way in which the primordia initiated by the apex develop. The rates of primordium initiation and apical growth decrease on reversion, but remain markedly higher than in the original vegetative apex. *I. balsamina* can therefore have two types of vegetative apex, each characterized by a particular primordium arrangement, rate of primordium initiation, and apical growth rate. These differences between the apex of the reverted and the original vegetative plant imply that there have been changes at the cellular and molecular levels. This might suggest that the apex on the reverted plant has been altered in such a way that its subsequent response to induction may be different from that of the original vegetative plant. If the subcellular changes are some of those which normally occur during evocation then the reverted apex could be regarded as partially evoked. These changes would presumably be concerned with altering the metabolic state of the apex, since in the reverted apex they are clearly not correlated with the formation of floral organs. By following cellular changes in the apex during induction and reversion it may now be possible to identify those partial processes of evocation which are specifically required for new types of organ to be formed, since we would expect these particular processes to be reversed during reversion of the apex.

At present we do not know how long the altered vegetative state of the reverted apex persists. However, the rate of primordium initiation appears to decrease from about day 20 onwards in the 5 SD+LD treatment (Fig. 2), suggesting that eventually the phyllotaxis and growth pattern of the apex may revert completely to that characteristic of the original vegetative apex. If this is so, we might also expect that eventually all evocational changes in the apex would be reversed and the apex would return to its original state.

There is evidence that the size of primordia at initiation decreases in flowers (Lyndon, 1978), and this is also found in *I. balsamina* (Table 4). Another very characteristic feature of flowers is a lack of internodes; this may occur either because the internodes are initiated but do not develop, or because on initiation they are much smaller than in the vegetative plant. This question does not seem to have been previously considered, although the absolute size of stem frusta (the future node plus internode) during vegetative growth has been measured (Sunderland and Brown, 1956; Berg and Cutter, 1969; Smith and Rogan, 1975). It has also been suggested that a change in the vertical spacing of primordia could be the crucial factor controlling the changes in phyllotaxis typically found at the transition to flowering (Schwabe, 1979). On flowering in *I. balsamina* the length of the frustum at initiation decreases (Table 5), and this is correlated with the lack of internodes in the mature flower. On reversion internodes eventually reappear, but we do not yet know whether, when this happens, the length of the frustum on initiation also increases, or whether it remains the same as in the flowering apex. Meicenheimer's (1979, 1982) measurements of vertical growth rate per plastochron during flowering in *Ranunculus*



and *Epilobium* can be interpreted as estimates of frustum length at initiation, and also indicate that this decreases on flowering. The evidence available is therefore consistent with the idea that the absence of the internode at maturity is a function of decreased frustum length at initiation.

In the case of the plastochron ratio, only those primordia on the apical surface have been used to estimate the area of the primordium at initiation, and this estimate therefore reflects only the changes in growth of the apical meristem proper. On the other hand, when estimating the length of the frustum at initiation from the relative vertical positions of primordia below the apical surface, we must remember that at some point below the apical surface the growth of the sub-apical meristem becomes important, and cells of the stem frusta begin to elongate and differentiate. It is at this point that internode elongation is considered to begin in caulescent plants (Esau, 1977). Our measurements of the relative vertical spacings of primordia are based only on those primordia less than about 150  $\mu\text{m}$  below the apical surface, and in interpreting these measurements as estimates of frustum length at initiation we have assumed that the tissues bearing these primordia belong to the apical meristem itself. Internode length at maturity is presumably also dependent on factors operating after initiation, such as photoperiod and concentrations of growth substances, which alter activity of the sub-apical, rather than the apical meristem (Sachs, 1965). Further measurements of vertical growth in *I. balsamina* show that in vegetative plants the exponential pattern of growth per plastochron continues in tissues more than 150  $\mu\text{m}$  below the apical surface but that in flowering and reverted plants growth rate per plastochron declines in these tissues. This suggests that growth of the sub-apical meristem is less than in vegetative plants, and that mature internode length is dependent on this growth as well as on the length of the frustum at initiation.

Final internode size may also be controlled by the duration of internode growth. Investigation of this in relation to flowering and reversion would be of interest, since the final stages of cell elongation in the differentiating internode are known to be prevented by leaf removal, probably as a result of decreased auxin supply (Sachs, 1965). It may be, therefore, that one reason for the lack of internode development that accompanies flowering is the presence of floral parts (rather than leaves) which may not supply auxin needed for cell elongation in the internodes next to them.

The length of a stem frustum at initiation is closely related to the area of its associated primordium at initiation. Compared with the original vegetative plant, during flowering and reversion the sizes of the primordium and the frustum at initiation are smaller, both relative to the generating tissue and in absolute terms (Tables 4 and 5). The apical area growth rates and the vertical (frustum) growth rates are also higher in the flowering and reverting apices than in the vegetative apex (Table 6). These similarities between the growth of the apex in generating primordia and frusta may indicate that the generation of both is controlled by the same physiological processes. However, the initiation of the frustum (defined here as the time it first becomes visible) occurs at a variable time after primordium initiation. In the vegetative plant it occurs when the primordium is three plastochrons old (there are three primordia on the apical surface), whilst in flowering and reverted plants it occurs when the primordium is between seven and 10 plastochrons old. This variation in initiation time of the frustum presumably results from differences in the growth rate and number of cells allocated to the frustum at the time that it is determined. This determination may occur before the frustum becomes visible, as in the case of the leaf primordium which is determined before it becomes visible and therefore recognizable (Snow and Snow, 1933).

An unusual feature of flowering in *I. balsamina* is the lack of increase in apical area in the flower (Table 4). Further measurements have shown that the apical area also remains constant during the actual transition to flowering. In most, though not all, other plants the apex does increase in size on flowering (Bernier *et al.*, 1981). The attainment



of a critical apical size has been modelled as a causal event bringing about flowering in *Chrysanthemum* (Charles-Edwards *et al.*, 1979). It may be, therefore, that the lack of increase in apical size on flowering is linked to the ability of *I. balsamina* to revert. It would be of interest to know whether other species showing flower reversion (e.g. *Anagallis arvensis*, *Pharbitis nil*) also retain a constant apical size on flowering.

# ACKNOWLEDGEMENTS

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